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Title:

Study of the effect of lignin to a nanocellulose film

Author:

Alex Bernaus Johnson

Studies:

Degree in Industrial Technologies Engineering

Director:

Cristina Valls Vidal

Co-director:

Blanca Roncero Vivero

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Thank you's

Throughout this project I have received a lot of support from the whole Celbiotech laboratory at UPC. Both through guidance, with the use of equipment, lab techniques and information that has made this study a very educational experience for me. Cristina Valls, Blanca Roncero, Antonio Clemente, Oriol Cusola, Julia Fernandez, Albert Carcereny and Aina Cuixart have been the ideal day to day assistance that I needed to advance in this study. Thanks to their help, as well as the backing of family and friends I am proud to present this study.

Nomenclature

CA	Contact angle
CNC	Crystalline nanocellulose
CNF	Fibrillar nanocellulose
C	Control with sorbitol added as a plasticizer.
CN	Control with sorbitol added as plasticizer as well as NaOH.
Grammage	Mass per unit of area of a paper, also known as paper density.
LD	Lignin DP389
LF	Flax lignin
LFN	Sample with sorbitol and flax lignin obtained through addition of NaOH.
LS	Sulfonated lignin
LSU	Sample with sorbitol and sulfur lignin obtained through sonication.
PS	Polystyrene
Smoothness	Surface smoothness
T	Identification of intial test samples
TC	Control sample.
UV	Ultraviolet, divided in subtypes A, B and C.
WDT	Water drop test
µL	Microliters
µm	Micrometers

Throughout this project, nomenclature related to test samples will have a numerical index next to the code, this value indicates the concentration of lignin in that sample. For example, LS10, implies sulfonated lignin at a 10% concentration.

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Summary

Cellulose is the most common material in nature, that is mostly used in the production of paper. Studies with this product have developed a series of sub products that have interesting properties in a variety of applications. One of these materials, obtained by chemical or mechanical processes is crystalline nanocellulose, or CNC.

CNC, since it is a derivative of cellulose, holds most of its organic properties, but also allows a lot of flexibility in how it is processed. If it is disposed in thin layers and left to dry out for a period of time, it generates a thin film. This material resembles a plastic film, with the added factor of being slightly iridescent to light. Since this product has very similar properties to cellulose, adding a variety of other materials could affect its properties and allow for the creation of a film alternative. The greatest advantage of using such a product is the fact that it decomposes rapidly and doesn't use petroleum in its production.

Lignin is another sub product of the paper production industry, since this material gives paper a yellow shade that is usually not desired. Paper production plants use the lignin extracted in the pulping process to generate power by burning it down, but this material holds a very interesting property for making a film substitute. By mixing nanocellulose and lignin, it is possible to generate a film with different properties. This study intends to analyze these properties and evaluate if they could allow for this product to be an alternative to petroleum-based films. Other additives, besides lignin will also be used to allow for better sample testing, properties, or simply to allow lignin to fully dissolve in CNC.

Studies in this subject, and specifically in the use of CNC have been a growing trend in an attempt to reduce the manufacturing of petroleum-based materials. Throughout this study, physical, barrier and optical properties will be evaluated in relation to the concentration of lignin and other additives used in the production of films.

Aim

Analyze the effect of adding a natural biodegradable material, such as lignin, to the mechanical and optical properties of a crystalline nanocellulose film. Through this study, an in-depth comparison of different properties will allow to evaluate if lignin is an additive that can help generate high quality films that could eventually substitute petroleum-based plastic films.

Scope

This study will focus on the effect of lignin on the properties of nanocellulose films, as well as other additives used to allow for proper adhesion of lignin to samples. The following paragraphs detail what elements of study were essential in order to compare and properly characterize a film.

To begin with, a key part of this study was the analysis of existing data on the topic. Papers, studies and different types of published work were used to identify how this process could be more effective, and therefore obtain conclusive results. This step involves finding ways to generate films that could be used in testing, as well as additives that could bring positive properties to the sampled products.

Once films had been produced, testing of the different properties would allow to identify the effect of adding lignin to samples. In some cases, adding lignin also involved adding other materials, which meant the effect of these substances also had to be evaluated. Initially the properties that were measured were:

- Thickness
- Weight
- Brightness
- Transmittance
- Absorption
- Permeability
- Smoothness
- Contact angle
- Water drop test
- Transparency

The nature of this study implied that once properties had been analyzed, a following round of production and then testing had to take place, in order to follow the results that had been more effective in comparison to a petroleum-based plastic.

Basic Requirements

This study required a variety of different resources, but due to its nature had few limitations. Since the main reason behind this study is to find a biodegradable film with plastic like or superior properties, the main requirement was to make sure the materials used are completely biodegradable. Besides this important aspect to keep into consideration, the limitations of this study were set by the equipment the lab disposed. Testing certain properties, such as the biodegradability of a film is a long process that takes around three months, therefore certain properties were also subject to a time constraint. Since this was a key limitation, and it could not be tested directly, all materials used throughout this study were biodegradable.

In order to generate films and start this whole study a basic know-how was a key requirement. This knowledge was given by the UPC paper engineering Cellbiotech Laboratory, which included key information about the materials used, and the processes to follow in the study. The use of certain technology used to test the properties of films was also given by this group, which was an essential requirement for this study.

Following is a list of the equipment required to test the properties of samples:

- Mettler P163
- Thermo Scientific Spectrophotometer
- Technidyne Color Touch
- Bendsten Smoothness and Porosity Tester
- Universal Micrometer Frank 16502
- Infinity X microscope
- Data physics contact angle includes camera
- Carl Zeiss specular gloss
- Digital multimeter

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Justification

One of the greatest problems my generation will face is the ever-growing demand and use of plastic materials. Petroleum-based plastics offer extremely versatile properties at a low market price. This low market price is attached to a high environmental cost. We generate over 300 million tons of plastic every year, with approximately 50% of all this production pertaining to single-use plastics. As a society, we haven't grasped consciousness of the environmental cost the use of single use plastics comes with.

Finding biodegradable alternatives to the widespread petroleum-based plastics offers a solution that could reduce the negative effect we are having on the environment. Single use plastics are one of the main sources of concern, as their lifespan as a product is so short, but their effect on the environment is relatively large. This study intends to generate a nanocomposite from biodegradable materials. By trying to achieve the versatility and properties of a plastic film, hopefully one day single use plastics can be replaced by biodegradable materials.

The opportunity of generating a material capable of offering and competing with a plastic while being environmentally friendly was the main reason behind this study. Going to the supermarket and buying food implies all products are sealed, packed and carried in petroleum-based plastics. We often consider recycling a valid solution to this problem but recycling alone isn't enough to compensate for the volume of plastic generated. It is essential that we reduce the amount of plastic production and use drastically in the following years, if we hope to be sustainable.

Reducing the amount of plastic that is generated can only be achieved by offering competitive alternatives. Studies like this one hope to give more information on new materials that could one day drastically reduce our impact on our environment.

Personally, this project implied I could be in direct contact with an elite laboratory team, while studying a new nanocomposite that is aimed at reducing petroleum-based plastics. This meant I would have the opportunity to study and develop a solution to a problem in our society, and this is the reason why I choose to become an engineer.

1. Introduction to biorefineries

Cellulose is the most abundant organic compound on earth, as it is a key structural component in the cell wall of plants. This polymer, with lignin and hemicellulose forms the microstructure of wood. It is considered the basic building block of wood since it allows for the formation of the long tubular fibers that give wood its structure.

The fibers are mostly made of cellulose, but also contain hemicellulose and lignin. Lignin is most commonly found surrounding these fibers and keeping them stuck together. In figure 1, the long tubular fibers can be observed perfectly, where lignin is in charge of keeping these fibers together in a packed manner. From this visual, it is clear that wood is very different depending on which direction we consider. This is the reason why it is an anisotropic material, as the direction of the fibers will change its properties. Wood is considered a natural composite material composed of cellulose fibers and a lignin mesh.

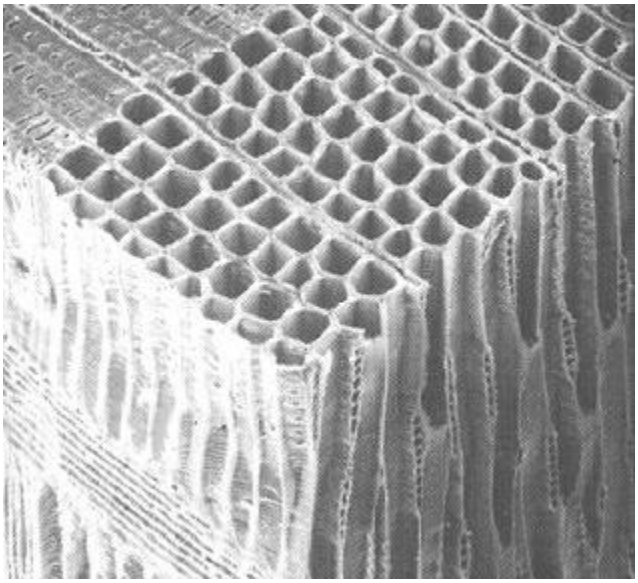

Figure 1: SEM image of wood microstructure

The use of these natural materials, which are considered wood sub products, is already very extensive. In the paper industry, cellulose and hemicellulose are used to generate all types of paper. Starting with wood chips, different processes are applied to achieve a lignin free paper. Lignin is extracted from wood to achieve a white, high quality paper that doesn't change in color

over time. Depending on the paper the mill intends to produce, factors like the process and the type of wood used are essential.

The production process of most common papers involves the extraction of lignin to keep cellulose and hemicellulose together, in a white colored pulp. Paper mills began using residual lignin as a fuel, due to its' high caloric value, to help generate energy to produce more paper. Energy is achieved by burning down this lignin, a process that allows a closed cycle and a far more efficient paper mill.

Burning down a material also has an environmental effect and is not the best way to use these residual products. The concept of generating products from compounds that are not used or are extracted in a process, would allow for a more sustainable approach to our use of resources. This is the concept of a biorefinery. A biorefinery is a facility that integrates biomass conversion processes and equipment to produce fuels, power, heat, and value-added chemicals from biomass (Silva et al. 2019). Today, a variety of products can be generated from the residual biomass of industrial processes. Studies are conducted worldwide to find ways in which industrial processes can generate more and be more efficient, by giving better yields or the production of sub products.

1.1 Nanocellulose

The market for nanocellulose is increasing due to the growing demand for biodegradable materials. Nanocellulose has a wide variety of possible applications that are currently being studied and even tested. The reason for this is that nanocellulose offers interesting barrier properties, optical properties and tensile strength with a very low weight. This material doesn't only offer intriguing properties, it is obtained from the most abundant polymer in nature, cellulose. Starting with pure cellulose and with a variety of different process two types of nanocellulose can be obtained, fibrillated nanocellulose (CNF) and crystalline nanocellulose (CNC). These two types of nanocellulose could have a variety of future applciations.

In a world concerned with decreasing the use of fuel, and in search of more efficient transport methods, reducing the weight of packaging and the transport methods themselves is essential. This trend is already present in some of the world's largest companies, such as ford that stated that it could reduce the weight of a vehicle by 340 kg by generating various components from nanocellulose (Nelson and Retsina 2014). The possible applications for these materials are numerous, and they keep on increasing as the process to obtain nanocellulose is simplified. Technological companies have developed an interest for nanocellulose based conductive materials, which could lead to a variety of innovations in electronics as we know them.

With the market for nanocellulose increasing and the technology era in full growth, the properties of this material are being used in very diverse applications. Studies have used

nanocellulose as an alternative to petroleum-based plastics in applications such as 3D printers (Klar et al. 2019). The biodegradable alternative presents ecological advantages, as well as the endless possibilities of generating composite materials. As mentioned previously this nanocellulose has been developed into a conductor to allow for electronic circuits, and with the addition of new technologies, these circuits could be 3D printed out of a biodegradable material.

Possible applications keep on surfacing as more knowledge is gathered on the properties of CNC and CNF. Studies mixing nanocellulose with non-biodegradable materials have developed lighter composites that are well suited for many purposes. Studies show films generated with CNF and non-biodegradable additives, can have properties that are comparable or even higher than those of petroleum-based plastics (Shimizu et al. 2014). For the development of projects that take this one-step further, by finding completely biodegradable nanocomposites, the production of nanocellulose has to be more affordable.

1.1.1 Nanocellulose production methods

Nanocellulose is obtained from pure cellulose. Even though cellulose can be found as a component in any vegetal source, obtaining pure cellulose is a complicated process itself. Due to the low current demands of nanocellulose, it is usually developed in pilot plants where high cellulose crops are used to facilitate the process. With an increase in interest for this new material and its broad range of possibilities, studies in new production methods were recently capable of breaking the cost barrier. Eventually, with new plants and a growth in the demand of this product, cost of nanocellulose should drop. Recent advances in this subject have developed different processes with the main objective of finding economically efficient ways of generating nanocellulose (Trache et al. 2017). These processes can be divided in two basic groups, chemical and mechanical production methods. Usually, chemical methods are used to produce nanocrystalline cellulose (CNC) whereas nano-fibrillated cellulose (CNF) is produced through mechanical methods.

Nanocrystalline cellulose production usually uses an acid hydrolysis step that will breakdown cellulose into a crystalline structure. This process, also used in the paper industry, uses an acid with the ability to give a positively charged proton to tear apart a bond. In the case of cellulose this results the separation of a crystalline and an amorphous region. The crystalline region is composed of structures that are approximately between 5-20nm. wide and can be up to 2µm. in length. CNC structures have crystallinity values between 65-90%, which implies that the way they are disposed repeats regularly in an ordered tendency. Crystallinity has a very important effect on a variety of properties of a material, specifically hardness and density. The process used to obtain this product will change the resulting materials' properties. Cost of CNC production is elevated due to the high chemical costs, water usage, and time cycles necessary.

Nano-fibrillated cellulose is obtained through the use of a homogenizer. Several companies worldwide have pilot plants destined to the production of this material. The use of a mechanical process to break down cellulose microfibrils into nanofibrils results in entangled fibers that are approximately between 20-40 nm. wide. The resulting fibers are longer than CNC and form amorphous long branches. Some methods to obtain this product use chemical pretreatments that allow for less energy consumption in the mechanical breakdown of fibers. Using less mechanical energy also implies that heat in this process is not as much of a concern, as it could affect the resulting material. Pretreatments using enzymes, such as endoglucanase, and TEMPO mediated oxidation are other alternatives in this process (Peretz et al. 2019). CNF production methods allow for a cheaper final product than in CNC production.

Recently, a new production method called AVAP® by the American Process Inc. has developed a biorefinery to generate both CNF and CNC at a commercial level (Nelson and Retsina 2014). This process uses sulfur dioxide as well as ethanol in a pretreatment to eliminate hemicelluloses and lignin from biomass. The mixture is then separated, chemicals are reused, lignin is used as fuel and other byproducts are used for biofuel production. Pretreatment stage of the process can be tuned in order to reduce or increase the amorphous regions in the material, which in turn affect the final amount of CNC or CNF produced. This process can be used with a broad range of biomass, is highly efficient in reusing byproducts and allows for clients to tune the resulting materials to their needs. With processes like this, nanocellulose production can be more cost effective, making it more suitable for future clients.

1.2 Lignin

Lignin is an essential component to the plant cell wall that provides structural rigidity to the plant's structure. Therefore, this organic compound is also an abundant product in nature. Lignin is a polymer with varying structures, that is recognized by a strong "wood" odor and a dark brown color (Figure 2). Lignin is in charge of keeping cell walls stuck together, as well as giving them rigidity, and the reason why wood has a brown color. This substance is also hydrophobic, which explains why even if wood is mainly composed of cellulose, which is highly hydrophilic, water is not absorbed as quickly in wood. Lignin was selected as an additive for films in this study since it is biodegradable, hydrophobic, is naturally found with cellulose in wood, and studies have proven it is an efefctive additive in films (Zhao et al. 2019).

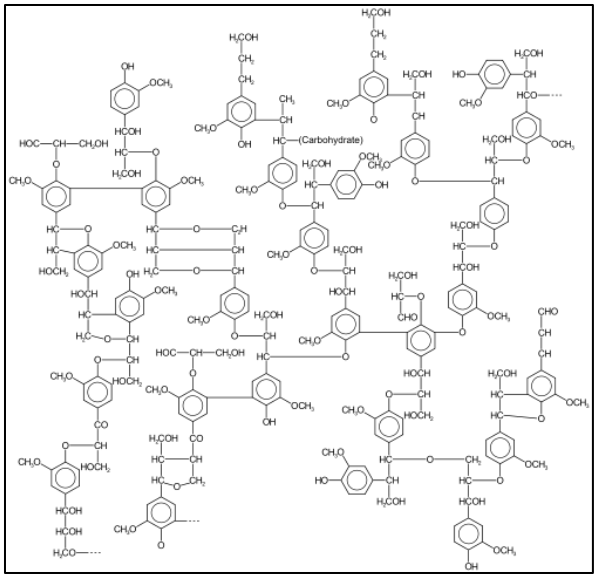

Figure 2: Lignin structure

Lignin is mostly considered as a byproduct from the paper industry, where it is usually extracted from pulp to achieve a white color in the final product. This process of extraction, used to whiten our resulting product, is done in a variety of methods. The extracted lignin is usually burned to generate energy for the plant to reduce the cost and energy consumption. Studies have analyzed the contamination the burning of lignin generates in areas where paper mills function (Pflieger and Kroflič 2017). The concept of a biorefinery intends to use this product not only as a fuel for paper mills, but perhaps to obtain other products. Obtaining energy from other renewable sources of energy would be an alternative for the paper industry which would also benefit in its' productivity. In many paper mills, the process of lignin extraction implies a queue time for other processes. It is essentially a bottleneck since this process takes time, while the whitened pulp waits for the plant to generate energy to continue its' process to become paper. Contamination problems, as well as productivity problems could be solved by finding alternative uses to resulting lignin.

Uses for a polymer such as lignin have been in development due to its unique properties. Studies have used lignin as an additive for biodegradable starch films due to its properties and that it is biodegradable (Bian et al. 2018). These studies have shown promising results in specific properties. The advantage in the use of lignin is that it is a byproduct of a specific industry, therefore methods to extract it have already been established and function today.

1.2.1 Lignin extraction methods

Lignin can be obtained from a variety of sources and through different processes. Due to this, the resulting substance can have varying properties. This is very interesting when it comes to studies that use lignin since, by varying the source or process through which it was obtained it is possible to obtain different properties. Throughout this study three types of lignin are evaluated in the films LD, LS and LF. A clear example of the varying properties in direct relation with the method used to extract the substance is the reaction these substances have to water. While mixing LF with water required other methods, LS would dissolve as soon as it was mixed. LS was still a very interesting candidate for this analysis, as studies have shown its potential in similar applications (Qian et al. 2011). In industries today two general methods are used to extract lignin form wood in the process of making paper. These two methods, named kraft and sulfite, generate two different types of lignin kraft lignin and lignosulfonate respectively.

The kraft pulping method is used to extract lignin by using sodium hydrixide and sodium sulfide. This chemical mix is known as white liquor, and together with specific temperature and pressure conditions allows for lignin to be extracted from the pulp. The remaining lignin is mixed in with the chemical compounds in a solution called a black liquor. Paper mills that use a kraft process use black liquor to recover the chemical solution and burn lignin for energy. This process is used in about 80% of the paper mills, since it reuses chemical compounds as well as byproducts, and has a high yield in the the pulp produced.

The sulfite process for lignin extraction was a lot more popular in the early 20th century, when recovery boilers had not been invented, which allowed kraft mills to recover chemical compounds. This process uses sulfites with different cations in order to break down lignin. The resulting lignin from this process is known as lignosulfonate, a polyeclectrolyte polymer. This means that this polymer dissociates when it is mixed in water. Interest in sulfite process paper mills is growing since this byproduct, lignosulfonate, has a growing interest for many applications. Uses for this compound range from an additive in cement, giving a higher resistance with a reduction of water use, to the production of vanillin, an artificial vanilla flavoring which is usually obtained in a petroleum-based process.

Alternative methods to obtain lignin also exist, such as using enzymes on rotten wood to extract lignin (Zavarin et al. 1982). These processes are not common in the paper making industry and do not generate lignin in large quantities. Both kraft and sulfite processes are used in paper mills today, and could allow for massive amounts of lignin as a waste or byproduct of paper production. Alternative uses to extracted lignin are already a growing trend, as can be seen by the growth of application for lignosulfonates.

1.3 Plasticizers

Plasticizers are substances used to increase a material's ability to deform non-reversibly under the effect of a stress. These substances are found either in liquid or solid form. Plasticizers also reduce a substance's viscosity and are used industrially in a variety of products. In studies using films, the use of plasticizer's, such as glycerol, has allowed for samples to gain properties that resemble the properties of a plastic (Miranda et al. 2015). Once a plasticizer has been added to a film solution, the resulting films resembles a plastic both physically and visually.

In this study, the plasticizer used was sorbitol. Sorbitol allowed for films to be manipulated, as well as other properties that are analyzed throughout section "3". This substance was used with all films since it allowed for films to be flexible, easily extracted and most importantly did not affect their biodegradability. Initially films were generated without the use of sorbitol, but these films were brittle to contact and hard to extract from their mold. Sorbitol was added to all films from that point on due to how it affected the resulting films.

Sorbitol is a sugar alcohol that can be produced by industrial processes, usually from corn syrup, or is formed in nature. It is used as a sweetener in many products as it has less calories per gram than sugar. Besides being used in the food industry, it is also commonly used in medical applications. Sorbitol and another plasticizer, known as Xylitol, were revolutionary in certain industries as they replaced sugar and allowed for a more hygienic solution. Sorbitol has been proven to reduce the growth of bacteria in comparison to normal sugar, generating an alternative in products such as gum. Sorbitol sweetened gum has a tendency to protect against bacteria causing periodontitis, the leading cause in teeth degradation in adults (Rafeek et al. 2019).

For this study, sorbitol was used since it can increase the plasticity of a film. This fact allowed for an easier manipulation of samples throughout the whole process of testing. The properties that sorbitol added to the sample films are thoroughly analyzed as it was an essential part of this study.

1.4 Sodium Hydroxide

Sodium Hydroxide, also referred to as caustic soda is used in a variety of applications. It is formed by a sodium cation and a hydroxide anion, forming the chemical formula NaOH. This compound is characterized by being a corrosive base that can easily dissolve in water. It is commonly used in cleaning, treatment of water and even chemical pulping due to its' properties. Another interesting use to generate aluminum from bauxite, the state in which it is naturally found. Industrial use of caustic soda is very common as can be seen by the range of applications of this alkaline substance.

In this study sodium hydroxide allowed to dissolve LF in the solution when mixing was not sufficient. This compound has a strong alkaline PH which is used to dissociate lignin in kraft process pulping. This same effect allowed it to dissolve, forming a homogenous solution, in the samples tested. Studies have evaluated the effect of NaOH in films of a similar nature to the biodegradable samples tested, these effects are evaluated throughout this study.

1.5 Film properties

The properties tested in this study allow for a comparison between the lab produced samples and products that exist in the market. The properties that were chosen are essential in the function of a film and will show if adding lignin or other additives to the film can influence its' role as a future product. To clearly distinguish what types of properties will be evaluated throughout this study they will be divided in three major groups; physical, barrier and optical.

1.5.1 Physical properties

Physical properties often involve data that is relative to the sample itself, and not a measure of how this sample reacts under a specific condition. Within this group of properties, a variety could be used to analyze a sample of these characteristics. The three main properties chosen in this study grammage, thickness and smoothness are commonly used in the paper industry.

The study considers grammage, a measurement that is also called paper density, which is an indicator of the weight in comparison to the surface of the sample. Within this group of properties are also included the size, thickness and weight of each sample. These properties are directly correlated to the amount of solution that is used in the production of a film. Therefore, drawing conclusions from the possible effect of adding lignin to the physical properties of a film is bound to be unprecise. In any case, it is essential that these properties are similar to the market products we intend to eventually compare samples with.

The best measurement to clearly establish a comparison between available products and samples are grammage and thickness. In the case of thickness, an average paper is approximately 100 µm thick, while a plastic film is usually about 40 µm thick. Throughout this experiment the goal is to generate samples that are around 60 µm thick. Both in the case of an industrial film and a lab generated film, values for thickness will have variance levels. In the case of samples created in a laboratory, these are expected to have important values of variance. Industrial films are usually generated within parameters of tolerance. Films are tested throughout their production line to evaluate if they are within the tolerance expected, if they do not fit these parameters they are usually discarded or used to generate a new batch depending on the material. For lab generated films, they were discarded or not used for testing if their values didn't fit the range

expected. The focus of this study was to evaluate the effect of lignin and other additives necessary to dissolve lignin, not to evaluate the effect of thickness on film properties.

The last physical property analyzed was surface smoothness, which throughout this study will be referred to as smoothness. The effect of smoothness on a film is strongly related to the application of a film. For films with an aesthetic purpose a product design team might be more interested in a material that is smooth, while instead in product that need to be handled a better adherence could be of more interest. High end products take into consideration properties like smoothness in order to deliver a product that can be the best fit for that application. Therefore, the effect of lignin or other additives in CNC films will be measured, but completely depends on the final application of the film.

1.5.2 Barrier properties

Barrier properties, also called permeation properties, are essential in the characterization of a film, as they consider the ability the sample has of protecting from exterior effects. In the hypothetical case a film is protecting food, or any other product from contamination, barrier properties measure how this film will protect the product.

Barrier properties are the main reason why petroleum-based plastics are so commonly used, as they have outstanding barrier properties at a low production cost. The properties that are considered within this group include permeability, oxygen permeability and hydrophobicity between others. A film's structure has a crucial effect on how materials are capable of permeating it. In the case of plastics, long polymer chains that are tangled generate structures that are hard to permeate. Polyethylene, the most commonly distributed plastic in the world is also one of the ones with the simplest structure. It is composed of a variety of polymers of the ethylene group $(C_2H_4)_x$, joined together to form a plastic with high gas and humidity barrier properties. Ethylene polymers joined together offer these barrier properties due to the fact that they are tangled and form weblike structures. Therefore, an essential factor in the resulting barrier properties of a material is how it is structured.

1.5.3 Optical Properties

Optical properties consider how a film interacts with light. Most films in the market are transparent, but protection from certain wavelengths of the electromagnetic spectrum is a growing trend in modern investigations. Studies have shown ultraviolet light can damage products. Therefore, a possible way bio nanocomposite films could improve the properties of existing films is by protecting from ultraviolet light (Sadeghifar et al. 2017). This study will analyze optical properties such as transparency, specular gloss, transmittance and absorbance.

Optical properties allow both for an aesthetic analysis on the film manufactured as well as a functional analysis on the data gathered. Tests on these properties allow to give numerical values to visual characteristics that can be seen in each sample. As mentioned earlier, some optical properties are essential in the functionality of a film. One of the priorities within the study of optical properties was to analyze how varying wavelengths affect a films' ability to absorb light. Light that reaches the surface of the earth, also called solar irradiance, is a form of electromagnetic radiation that we have defined using the electromagnetic spectrum. In this spectrum, radiation is organized by its' level of frequency and is separated in different groups or bands. Light coming from the sun comes in three of these different groups, infrared, visible light and ultraviolet. Of these three groups, the highest frequency band is ultraviolet light. Since frequency and wavelength are inversely proportional, ultraviolet light has the shortest wavelength of these three groups and is the most damaging to living organisms.

Ultraviolet light, often abbreviated as UV, ranges between 100-400 nanometers, higher wavelengths correspond with visible light and lower values with x-rays. This range of light is also broken down in three different groups, UV-A, UV-B and UV-C. UV-C is the highest frequency of these values and ranges between 100-280 nanometers in wavelength. Since it has the highest frequency, it also has the highest energy, meaning it is the most dangerous to living organisms. All of this subsection of ultraviolet light is absorbed by the ozone layer in the atmosphere before it reaches the surface of the earth, protecting organisms from the effects of this radiation. UV-B has a wavelength between 280-315 nm. and is mostly absorbed by our ozone layer. Within the types of electromagnetic radiation that reach the surface of the earth, this is the most damaging type. UV-A ranges between 315-400 nm. and is barely absorbed by the ozone layer. Both UV-A and UV-B are the cause for a variety of diseases and skin damage on living organisms.

The effect of different electromagnetic radiations on studied films was essential since this would allow for them to be used in many more applications. Lignin has shown a tendency to absorb light around values of 280 nm. and could be an ideal additive to enhance this property. Studies trying to find ways to generate a film that can protect against light in this specific region have been trending. Some alternative methods evaluate additives such as carbon to allow for higher absorbance levels (Feng et al. 2017). Lignin has also been studied as a natural additive in sunscreens, to develop a product to protect skin from ultraviolet radiation.

2. Materials, equipment and techniques

This study used a variety of different materials as well as techniques, since the main objective was to evaluate the properties of a bio nanocomposite film. The resources used throughout this study were essential since they made up the composition of the samples being tested. Nevertheless, an important part of these samples was not only their composition, but the resources and processes that went into their production. Once film samples were produced, different techniques and equipment were used to measure properties of interest that would allow for further analysis.

2.1 Resources

The resources used throughout this study include the materials used on the films, or those the films are composed of, and the materials used to generate those films. Even though these are common lab materials, it is important to detail what was used in order to facilitate reproducing this study.

Materials used in the composition of sample films are detailed and introduced in "section 1", but the following table allows for a clear distinction between the nomenclature used throughout the project and the materials being used.

Main Resource	Plasticizer	Additives	Lignin	Nomenclature of sample
Crystalline Nanocellulose (CNC)	None	None	None	TC
			Flax Lignin 1%	TLF1
	Sorbitol 15% (Plasticizer)		None	C
			LS 1%	LS1
			LS 2%	LS2
			LS 10%	LS10
			LS 1%	LSU1
			LD 1%	LD1
			LD 2%	LD2
			LD 10%	LD10
		NaOH	CN	CN
			LF 1%	LFN1
			LF 2%	LFN2

Table 1: Different materials used according to nomenclature.

The materials of which the samples are made of include:

- Cellulose nanocrystals (CNC) provided and produced by University of Maine (Maine, United States)
- Sorbitol by Acros Organics BVBA (Geel, Belgium).
- Lignin LD, LF and LS which was provided by Borregard (Sweden).

Besides the use of the materials which the films were composed of, a variety of other materials were used to generate samples. Plastic molds were created that required polystyrene plastic plates, hot silicon guns, and plasticized paper. Polystyrene, PS, was a good material to generate the molds from since it is easily accessible, cheap, and did not stick to the solution. These molds were used to settle the liquid solution, which was left to dry between 7-10 days. Any other flat mold with enough size to allow for property measurement would have served the same purpose, but this way each sample had a similar size.

To create the liquid solution with the correct composition of the sample, well cleaned lab glassware was used as well as an agitator to keep the solution as homogenous as possible. Details on the actual process of film production are explained in section "2.2". Distilled water was used in the composition of the solution as well as a key element to clean and test the properties that required it. PS molds were cleaned with alcohol before and after each use to keep samples from being contaminated by dust or other particles.

2.2 Film Production

Crystalline Nanocellulose is a gel like substance that is dissolved in a water solution to maintain its properties before use. In order to obtain films to test properties that could compare to a petroleum-based plastic, a production method had to be designed. This process must be the same throughout the study, since the method used to produce these films could have an effect on its' properties. The samples being developed required a minimum size, since the properties tested used a specific size. The mold used to obtain these films had a surface area of 95 cm^2. This size, with a minimum of two samples produced of each combination of materials allowed for testing of all properties specified in section "1.5".

The mold was prepared from a PS plastic plate which was then modified by adding vertical 1.5-centimeter walls on the sides. These walls would contain the fluid solution from moving towards the sides and facilitate the extraction of the dried-out films (figure 3).

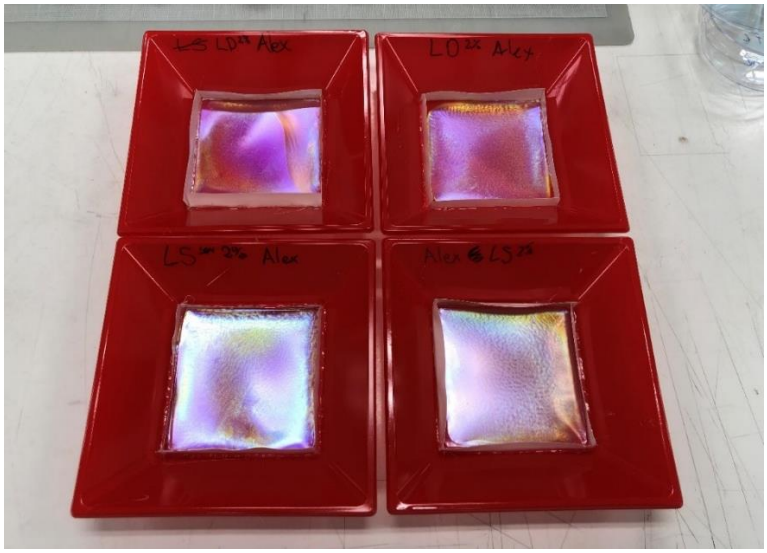

Figure 3: Molds used in film production with samples on them.

All films were generated in the exact same standard conditions; 23º C, 50% humidity room. They were left to dry until they were completely dry, in some cases even generating air cavities between the mold and the film due to their contraction as they went through the process of drying. Once they were completely dry, films were extracted from the mold by slowly pulling on the previously cut out section. Since the films were cut for extraction, their size varies slightly, as explained in section "3.1".

In the initial process of film production, samples did not contain a plasticizer, the immediate effect on the process was that the film was attached to the mold. Adding plasticizer to the production allowed for a more successful extraction, and full pieces of film being extracted at once. From this point on, plasticizer was added to all films generated. The specific amount of plasticizer added is calculated in section "5" and is based on an analysis of different plasticizers and their concentration completed by PHD candidate Julia Fernandez, a member of the Celbiotech group.

Once the addition of sorbitol as a plasticizer was definitive, samples with different concentrations of lignin were produced. The calculations for the amounts of all substances used, including lignin and NaOH, to produce these films is detailed in section "5", the calculations annex. The process of production was the same as had been established, to make sure the effect of lignin was being compared and not any effects the film production process could have on the properties of the films.

2.2.1 Flax lignin film production

Initially, while developing flax lignin films, this compound's high hydrophobicity implied it wouldn't dilute in a CNC solution. To prepare a film, the solution had to be homogenous, if not properties would vary depending on the section of the film tested. To achieve a homogenous solution, two possible solutions were found, the use of sonication and addition of sodium hydroxide.

Sonication uses ultrasonic frequency sound waves to agitate a sample. The results were positive; a 10 minute cycle allowed for the flax lignin to fully dissolve in the solution. Sonication was used in other samples throughout this study, and was considered as a possible factor for changing film properties. Comparisons were established between the effects seen in samples that were sonicated and other studies that evaluate how this process affects film behaviour and properties (Ding et al. 2019).

Flax lignin sample films were also obtained by adding sodium hydroxide to the sample. As explained in the section of additives "1.4", this substance allowed for flax lignin to dissolve and create a homogenous film. Sodium hydroxide used was a standard product obtained at the Celbiotech laboratory with 40g/L concentration of NaOH. The valume used in the process of film production was directly dependant on the amount of lignin added to the film. Films containing sodium hydroxide, reffered to with the letter N, and no lignin were also developed , sample CN, in order to analyze the specific effects this substance had to film properties. The effects of this substance on the film are analyzed in section "3" but were not a concern from the standpoint of film production.

2.3 Techniques and equipment for physical properties

Physical properties were the first to be tested in this study since they were nondestructive. The equipment used for these properties did not damage samples, and therefore it was best to take this data at the beginning of the testing stage. The fist properties tested were mass and size, measured right after the extraction of the sample from its' mold.

In order to measure the mass of a sample, a Mettler P163 balance was used (figure 4). This equipment is used in the paper industry to measure the grammage of different types of paper.

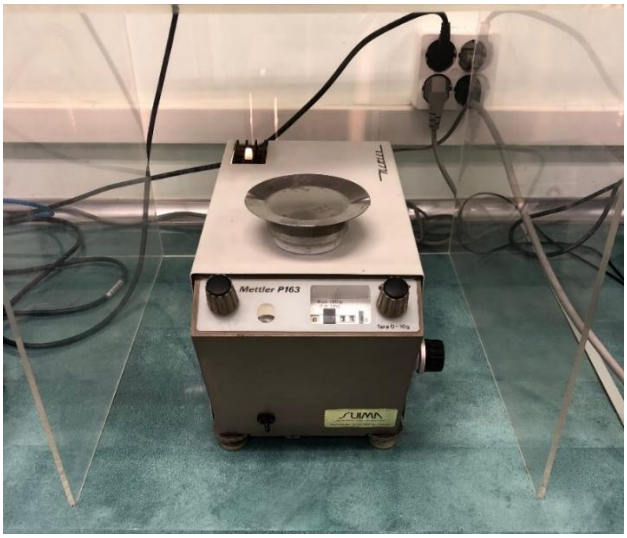

Figure 4: Mettler P163 high precision mass measurement equipment

The Mettler P163 uses a scrolling mechanism to find the correct mass of the film. A circular plate located above the balance is used to place the sample measured. In the laboratory, this balance is covered so the measurement is as precise as possible. The size of the film was measured using a ruler. Some samples were slightly irregular on the sides and were cut to allow for a more accurate size measurement. Data on size and mass allowed for the calculation of different values for the grammage of each sample.

Sample's thickness was measured using a Frank IDM thickness equipment (figure 5). This equipment was first calibrated using a specific norm. Depending on the type of material tested, different masses were added on the superior side of the machine. In the case of the samples measured, due to their values of grammage, the same norm (UNE-EN ISO 534:2005) used for paper was applied. Once the mass was set, the machine used an automatic calibration system. Once the equipment was ready, settings were choosen that allowed for repetitive measurements whenever the user required. In order to have an accurate measurement for the thickness of samples, various measurements were taken, since it is possible the film was not homogenous while it dried.

Figure 5: Frank IDM thickness equipment

The last physical property measured was smoothness. The equipment for this property used a flow of air over the sample surface. Once the sample was placed flat on a surface and the equipment (Figure 6) was placed over the sample, the flow of air generated would either spread or generate an upwards resistance. The measurement of the upwards resistance shows a value for the smoothness of samples. This equipment used a 150-gram mass to balance the equipment. Various measurements were taken in order to take into consideration the fact that the sample may be heterogenous.

2.4 Techniques and equipment for barrier properties

Barrier properties are essential in the function that is expected from a film. In this study, the hydrophobicity of a sample was an important property to measure due to the properties of lignin and nanocellulose. Barrier properties selected were air permeability and different ways to interpret the sample's behavior under the effect of water.

To test a films permeability to air, a test based on a norm was used. This equipment works by generating a flux of air over a film, the air that can't permeate the sample is then generating a force in the opposite direction (figure 6). This force is then measured by a small mass within a glass tube that can be regulated according to the flow of air coming in. The force is then compensated by a 150-gram mass.

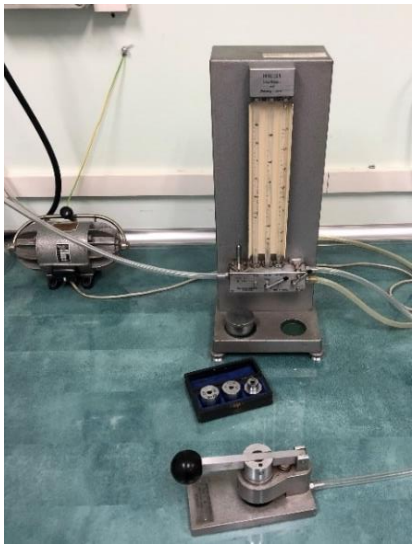

Figure 6: Equipment used for smoothness and air permeability

Films are often used to protect or cover products in situations in which they may be in contact with water. Due to this precise reason, it was essential to measure how hydrophobic the films tested were. Initially, a simple water drop test was measured in order to evaluate how long a drop of water took to be fully absorbed by the sample. A highly hydrophobic film would take a long time to absorb the drop, or perhaps would not absorb the drop at all depending on how hydrophobic it is. The water drop test, or WDT, is performed by resting a sample at a distance of 2.5 centimeters from a static syringe that is then actioned to deploy a single drop of water. Then, the time the drop takes to be absorbed is recorded (figure 7).

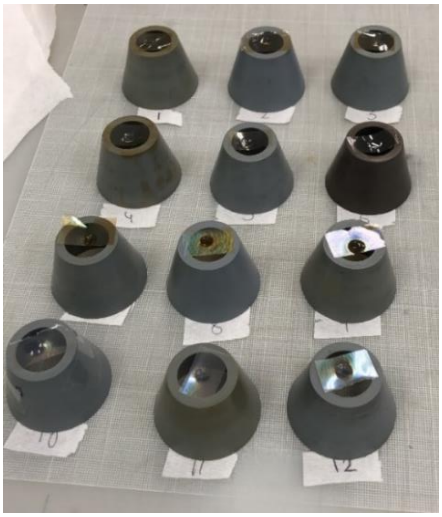

Figure 7: Water drop test.

As shown in the image, samples used were placed so they were only rested by the sides, so the area in contact with the drop was completely floating. In case the drop was to fall too close to the sides of the sample, the test was considered not valid. The norm followed for this test did not specify any conditions for the samples thickness or the size of the sample, only the distance between it and the syringe, which was of 2.5 centimeters. Due to the shape of the rubber stand used, the samples used were approximately 2.5 x 1.5 cm in size.

This initial water drop test was a good indicator of how hydrophobic a sample was but didn't provide enough information about this property. Therefore, two other tests were used in order to measure the hydrophobicity of each sample. Both tests used the same equipment and focused on the shape of the water drop when in contact with a sample (figure 8).

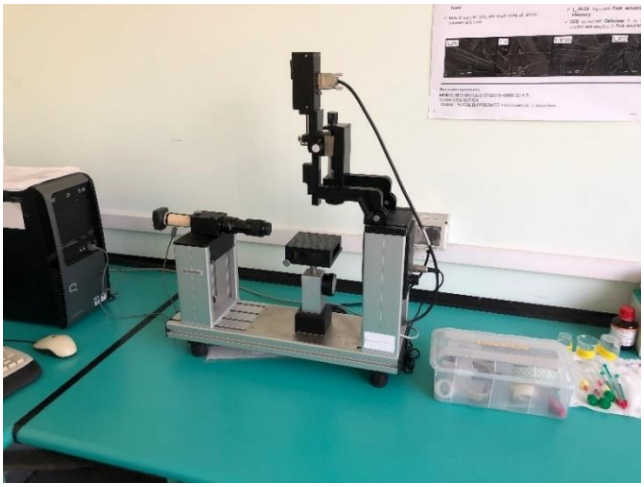

Figure 8: Micro syringe equipped with a high definition camera

Contact angle allows for the measure of the angle between the sample and the water exactly when it settles on the film. This equipment works with software that allows for a frame by frame analysis of a recorded set of data. By showing the software the area of the film, and the appropriate frame, it detected the drop and the angle that is generated between it and the sample. Analysis and process on this technique was compared with the published work of members of the Celbiotech laboratory (Cusola et al. 2014).

This equipment generates drops with a Hamilton 40µL syringe, which was actioned by the equipment to have the same volume of water disposed in every test. Proper measurements required the sample film is placed firmly on a device that allows it to float. This way, the bottom part of the sample is never in contact with another surface and the high definition camera can see a clear line where the sample is located which assured quality measurements (figure 9).

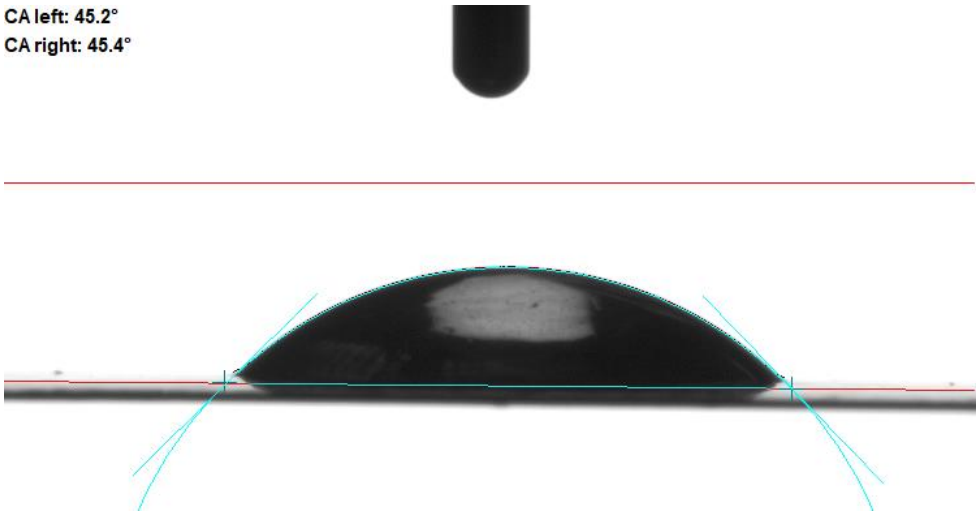

Figure 9: Image of software analyzing the shape of water drop over sample.

Once the parameters had been identified, the recording function analyzes how this angle changes in time. This data allowed for another test of the barrier properties of the film, an absorption curve. For this test, the software had to be set to analyze how the drop evolved over the surface of the sample. The initial moment of contact between the drop and the samples is considered "time zero" and is set by the user. The following sequence of images depicts how the user sees this transition frame by frame (figures 10 and 11).

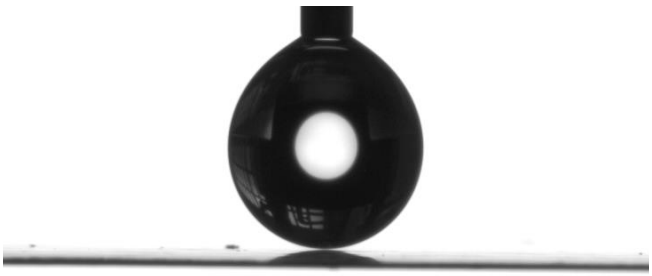

Figure 10: Frame before contact between drop and sample.

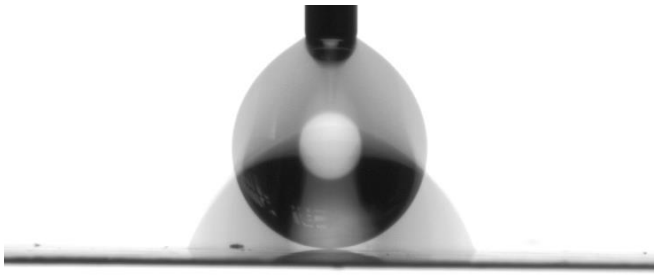

Figure 11: Frame of contact where both states are captured.

Measurements are collected once the frame shows a lucid drop, without vibrations or shades. This instant is used to collect the contact angle and to start measurements for how this parameter changes over time. An example of this precise moment is shown in the following image (figure 12).

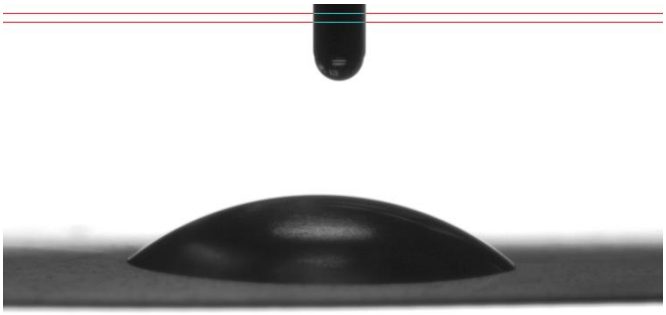

Figure 12: First frame where drop is clear, set as "time zero".

2.5 Techniques and equipment for optical properties

Testing of optical properties involved analyzing how sample films interact with light. In order to test these properties in the laboratory, a variety of different equipment was used. To begin with, tests that did not need to damage the sample were executed. Following these tests, a spectrophotometer was used to record measurements for absorbance and transmittance.

The first nondestructive test that took place was the measurements for specular gloss in samples. For this test, a voltmeter was connected to the device. The voltmeter measured the potential difference between two nodes, which were first evaluated under a white test probe and a black

test probe. Both of these probes are measured to have parameters from which to evaluate the measurements obtained. The black probe absorbs light emitted by the equipment, while the white probe should reflect all incident rays of light. The nodes measure the amount of light that is being reflected, allowing for a measurement for the specular gloss of a sample (figure 13).

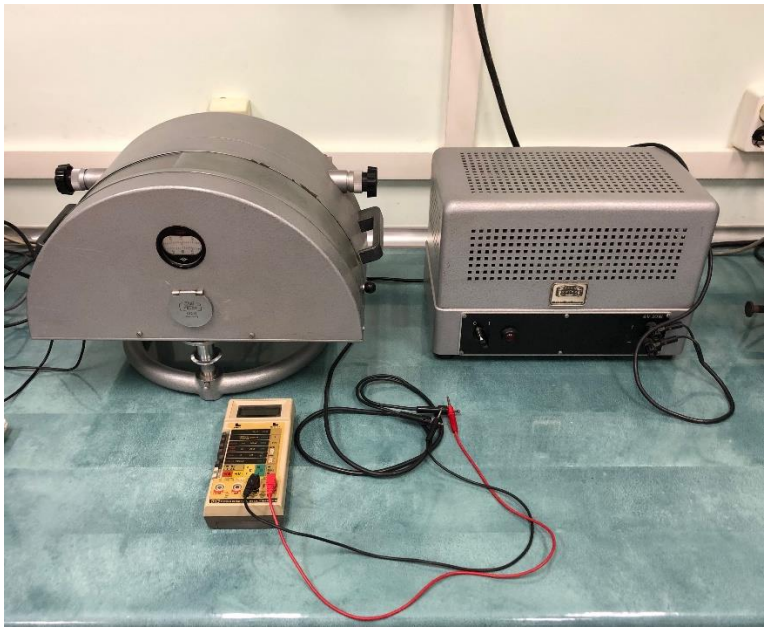

Figure 13: Carl Zeiss equipment for specular gloss

The incident rays of light could be calibrated, but the norm for this test establishes an angle of 20^{o} to measure specular gloss. Every time this equipment was used, a new white and black probe measurement was recorded to make sure the conditions were always the same.

To test a films transparency, a typical property in most petroleum-based films, a first test was conducted using the Technydine Color Touch equipment (figure 14). These measurements required a computer and software that would analyze and record the data measured. This equipment also required a series of measurements on a black probe as well as a control recording. The software measured the data and used formulas established by the norm to give a result for a sample's transparency. A separate data analysis tool, generated by the Celbiotech laboratory, was required to check the results.

Figure 14: Technydine Color Touch used to measure transparency

The use of a spectrophotometer was necessary to analyze how light with varying wavelengths interacted with samples (figure 15). In order to use the spectrophotometer samples had to be cut into 3.4 x 0.8 cm probes which could then be inserted into a quartz recipient. Containers to hold the sample were made of quartz, as this material does not interact with light and allows for very precise measurements. For this specific test, in which the UV light spectrum is of interest, a quartz container is strongly recommended instead of other options. Other materials, such as methacrylate, would have generated erroneous measurements in the UV section of the light spectrum. Samples in the spectrophotometer are usually diluted for testing, in this case the norm required the probe to be placed flat against the quartz surface. A first measurement was taken without any samples to generate a baseline for future data. While using this equipment touching the probes and the container was avoided so data was not contaminated by manipulation.

To test the absorbance of samples, a broad spectrum of light that included UV light was selected in the software. This application allowed for the spectrophotometer to turn on the according lights to generate wavelengths in the range demanded. For this study the light spectrum was between 200 and 900 nanometers, which included UV and visible light. In some cases, the spectrophotometer interpreted values with error, which could be identified by sudden noncontinuous values of absorbance collected. Data collection was repeated making sure the quartz container was clean and the sample was placed flat against the container's surface. Data was then collected and analyzed using data analysis software that was used to generate graphs for the results "section 3.3.1" of this study.

Figure 15: Spectrophotometer in Celbiotech laboratory

The spectrophotometer was also used to collect and measure data on the transmittance of samples. In this case, the value for the wavelength of the incident ray of light was set at 600 nanometers. The containers and methods used for this test were the same as to evaluate absorbance, but methacrylate recipients could be used since incident light no longer corresponds to the UV section of the light spectrum. Transparency was also gathered using the values obtained on the percentage of transmittance at 600 nanometers. Values were compared with those obtained through the use of the technydine equipment, figure14, and the data collected through the transmittance test was evaluated as more accurate.

2.6 Other techniques and equipment

In order to visualize the structure of samples, microscopy was used (figure 16). Microscopes in the Celbiotech laboratory are connected to software that allows for the visualization of the probe. This equipment allowed for magnification at different ranges. For the samples observed, a higher magnification than 200x was not visually clear. Therefore, the range of magnifications used in this study were between 4x-200x. This part of the analysis was used as a visual guide and was not done following a specific order or method. Specific samples were selected since they offered different properties.

Figure 16: Infinity X microscope

For the use of this equipment some samples were cut into pieces that were placed between glass in order to create probes for the microscope. The use of a color indicator, which would allow for the differentiation between lignin and cellulose fibers was also used. This was not affective since cellulose fibers were not visible, as they were broken down to CNC.

3. Results

Bio-nanocomposite films were the subject to the results explained in the following section. Throughout this section, films will be compared based on the substances used for their elaboration and the properties these will give them. It is important to note that as explained in the film production section "2.2", sorbitol was added to films after the initial batch of testing due to the effect it had on the samples. Films were initially brittle to contact and could not be handled with ease. Once the plasticizer was added to the material, the final samples became more flexible and manageable. This is the reason why some properties were not tested with the batch that didn't contain sorbitol. This plasticizer is considered vital in the resulting film and was added in the same concentration to all films from that point on.

Throughout the production process two films of each sample were manufactured, using the exact same process for each sample. Barrier and optical properties were measured using only one of these samples, labeled A or B, while thickness was measured for all films produced. Thickness was a control parameter for the films produced, if these values were very different, the film was

not used, or considered valid (C A). By following this procedure, testing the correlation between thickness and other properties was avoided, and the focus was on the effect of lignin and other additives.

The following table shows all the samples with sorbitol produced throughout this study to allow for a visual reference:

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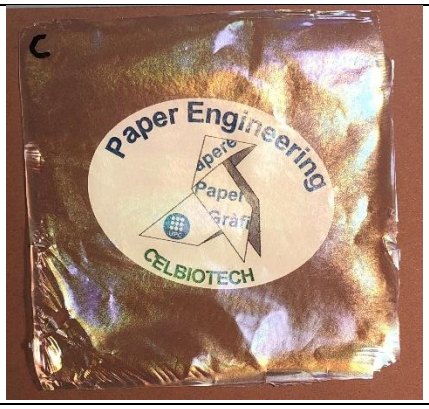	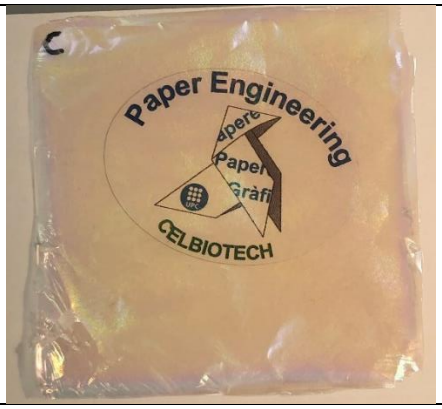
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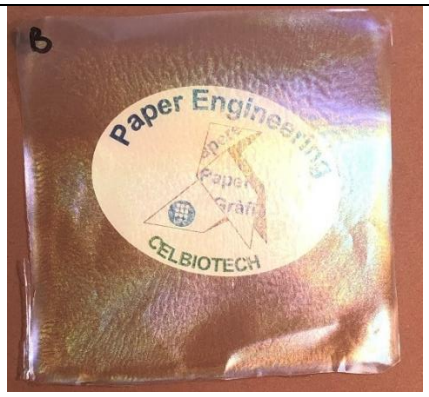	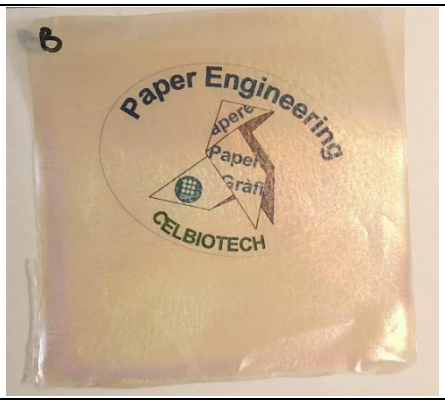
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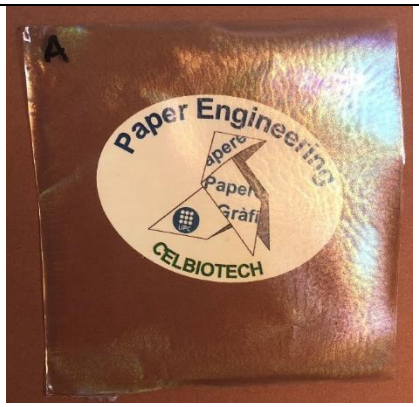	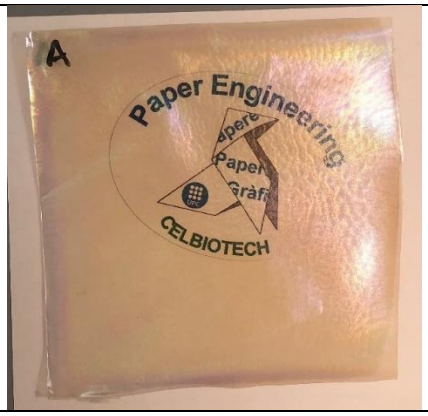
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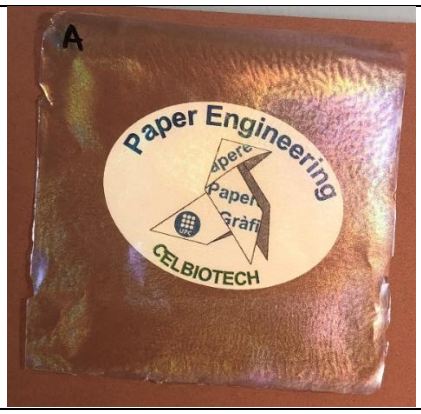	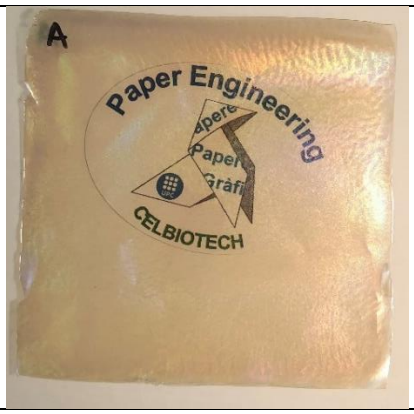
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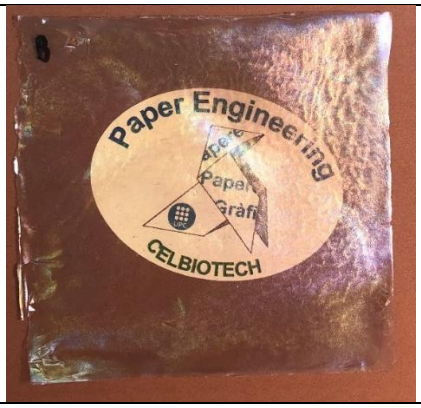	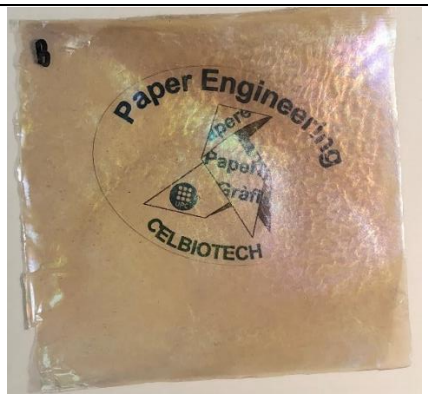
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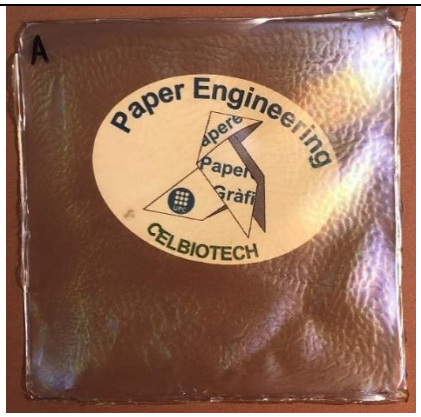	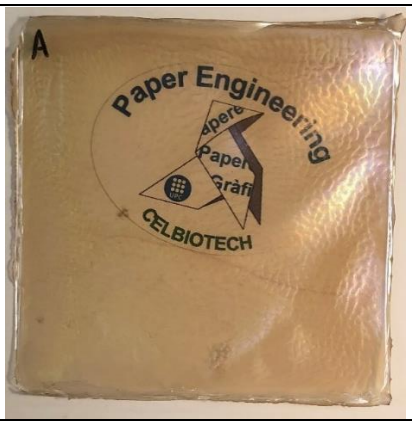
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	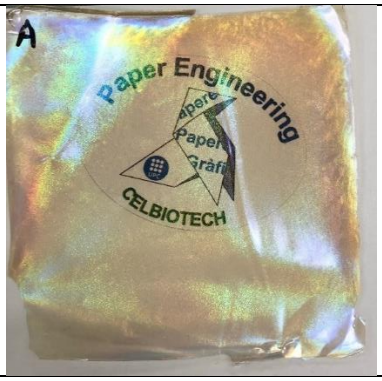

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	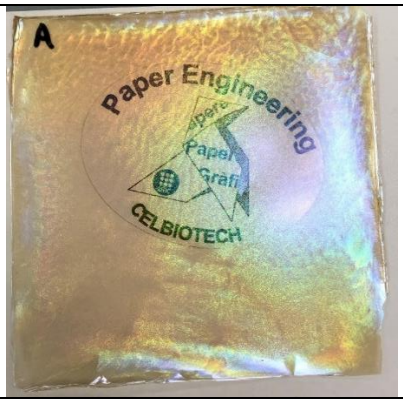
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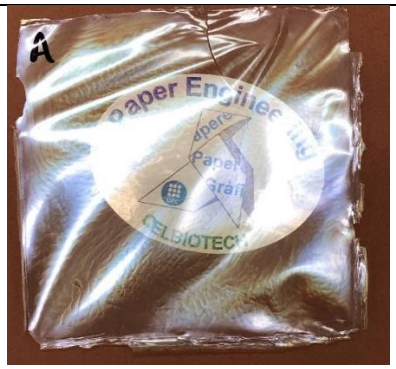	
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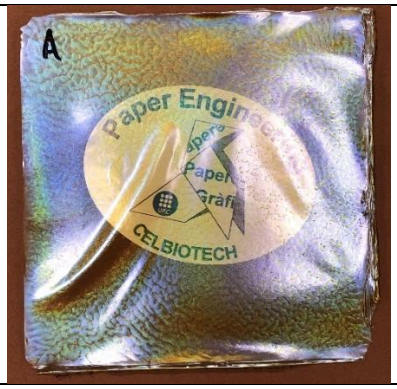	
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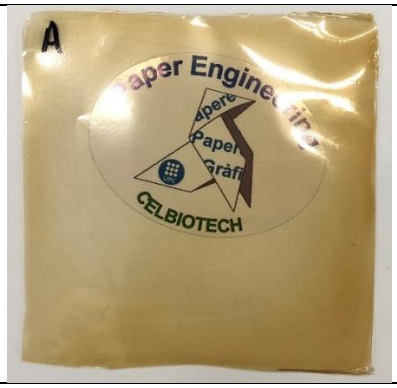
	

Table 2: Visual reference of films generated over different papers.

The data collected from measuring the different films produced is separated by the groups of properties that are essential in the functions that are required of a film; physical, barrier and optical.

3.1 Physical properties

Physical properties of films were the first to be measured. Data from samples generated throughout this study were considered for these properties as a method to evaluate if they could be compared in the following sections. If a sample had a grammage value that was much larger than others, or far away from the range expected, this sample could not be compared to the rest. Physical properties measured aspects of the state of matter in the samples, which considering the analysis in the study were the mass, grammage, thickness and smoothness of a film.

3.1.1 Thickness

A film's thickness is directly related to the amount of solution used to generate this film. The following table details the values for the thickness of different films used throughout this study:

Sample	Thickness (µm)
TC A	50 ± 6
TC B	56 ± 5
TLF1 A	76 ± 12
TLF1 B	74 ± 7
Addition of 15% Sorbitol	

Sample	Thickness (µm)
C A	97 ± 30
C B	61 ± 7
C C	41 ± 4
C D	42 ± 5
CN A	54 ± 19
CN B	45 ± 6
1% Lignin with 15% Sorbitol	
LF1 A	77 ± 15
LF1 B	77 ± 18
LFN1 A	66 ± 17
LFN1 B	64 ± 11
LS1 A	47 ± 7
LS1 B	52 ± 9
LSU A	44 ± 6
LSU B	55 ± 9
LD1 A	45 ± 2
LD1 B	50 ± 5
2% Lignin with 15% Sorbitol	
Sample	Thickness (µm)
LS2 A	56 ± 16
LS2 B	52 ± 9
LD2 A	47 ± 17
LD2 B	57 ± 9
LFN2 A	51 ± 7
LFN2 B	65 ± 13
10% Lignin with 15% Sorbitol	
Sample	Thickness (µm)
LS10 A	41 ± 7
LS10 B	60 ± 7
LD10 A	80 ± 22
LD10 B	65 ± 14

Table 3: Thickness of samples

The production method of films was created to achieve films that were in the range of 50-70 µm in thickness. If any of these films had a value that was far from the range established, it was not considered for testing. Variance was also taken into account, since as the tolerance values show, this property presented many irregularities. In most cases the variance is within the range of thickness expected, and small units have a tendency of generating more significant measurement errors.

The initial hypothesis was that for greater values of lignin concentration, the film's thickness would increase. The reasoning behind this was that lignin particles would settle throughout the film making it both thicker and more irregular. If lignin has completely dissolved in the CNC solution, this effect would not be as noticeable. In samples with lignin that required additives to fully dissolve this effect was expected to be more pronounced.

The values recorded show that the addition of lignin had no real effect on the thickness of samples. Differences between the thickness of samples are attributed to the amount of solution used to generate the film. With a dimension like micrometers, a slight addition of more solution could have a far more noticeable effect on this physical property. Units with these dimensions were also expected to generate a higher variance in the data collected. The difference in values of thickness is within the variance calculated for each sample. All samples except for a control sample with sorbitol, C A, were within the range that was estimated during film production and calculation. This sample was not used for property testing to avoid thickness having an effect over the properties of the film.

3.1.2 Size, Weight and Grammage

An initial measurement of weight and size for all samples with sorbitol was taken in order to evaluate the effect of lignin in grammage, which was obtained through calculation. The following table shows the values of grammage, or paper density, calculated for the films used throughout this study:

Addition of 15% Sorbitol			
Sample	Size (cm^2)	Mass (g)	Grammage (g/m^2)
C A	90.25	1.06	118
C C	90.25	0.52	57
CN A	85.56	0.68	80
CN B	91.20	0.68	74
1% Lignin with 15% Sorbitol			
LF1 B	87.40	0.78	89
LFN1 A	96.04	0.94	98
LS1 A	92.16	0.66	72
LSU1 A	89.30	0.58	65
LD1 A	92.16	0.64	70
2% Lignin with 15% Sorbitol			
Sample	Size (cm^2)	Mass (g)	Grammage (g/m^2)
LS2 A	94.09	0.88	94
LD2 A	93.12	0.67	72
LFN2 A	94.09	0.77	82
LFN2 B	87.42	0.69	78

10% Lignin with 15% Sorbitol			
Sample	Size (cm^2)	Mass (g)	Grammage (g/m^2)
LS10 A	91.20	0.61	67
LS10 B	85.54	0.76	89
LD10 A	91.20	0.87	96
LD10 B	89.30	0.76	85

Table 4: Comparison between size, mass and resulting grammage of samples

The different values for grammage oscillate between common values for a material of these characteristics. The addition of lignin doesn't have a clear effect in changing the size of a sample or its' mass, therefore not affecting the values for grammage. In samples with a higher concentration of lignin, grammage should be slightly higher since more material was added to the solution, which then didn't evaporate in the drying phase. This effect is similar to the expect effect for the thickness of a sample. With more additives being used, samples should have more mass, while size is only affected by the mold. Therefore, higher grammage was expected, but the mass of additives was not as relevant as the amount of solution being used to generate the film. A slight change in the measurement used, or the solution not being completely homogenous when poured, had more effect on the grammage than the additives used.

3.1.3 Surface Smoothness

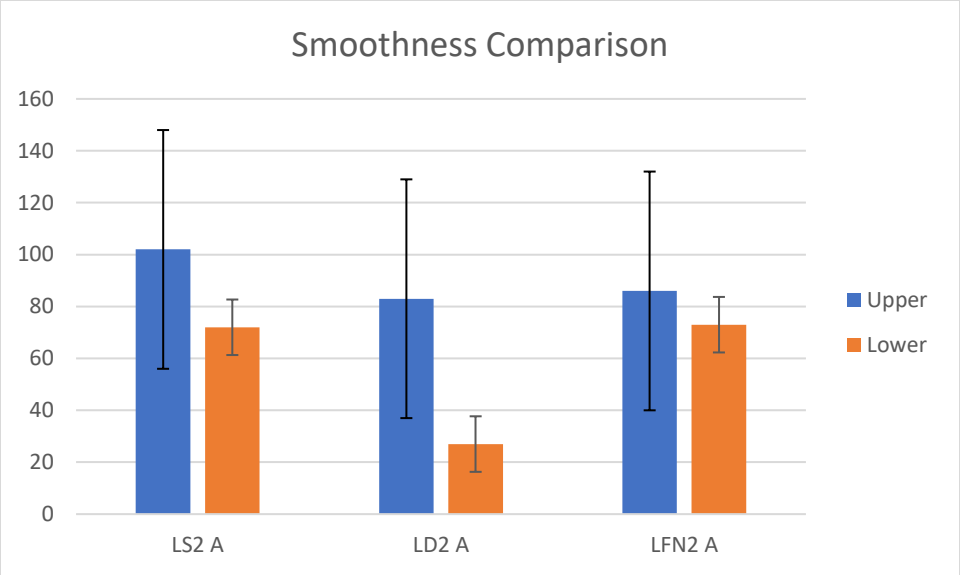
In the analysis of smoothness for a film, data from both sides of the film were considered. The reason for this, is that lignin granular structure could have sedimented while drying out, causing it to be in a higher concentration on the lower side of the film, and affecting the films smoothness. Perhaps, the fact that the film was not pressed into the mold and simply left to dry also meant that one side would be smoother than the other. To evaluate this effect, two series of measurements were taken for each sample tested.

Addition of 15% Sorbitol	
Sample	Bendtsen roughness (mL/min)
C C	88 ± 45
	32 ± 18
CN A	174 ± 72
	160 ± 130
CN B	42 ± 17
	60 ± 70

1% Lignin with 15% Sorbitol	
LF1 B	137 ± 67
	75 ± 22
LFN1 A	87 ± 26
	74 ± 39
LS1 A	124 ± 91
	36 ± 18
LSU1 A	144 ± 73
	63 ± 54
LD1 A	53 ± 18
	35 ± 29
2% Lignin with 15% Sorbitol	
Sample	Bendtsen roughness (mL/min)
LS2 A	72 ± 19
	102 ± 79
LS2 B	56 ± 16
	23 ± 4
LD2 A	83 ± 16
	27 ± 8
LD2 B	95 ± 15
	25 ± 7
LFN2 A	73 ± 5
	86 ± 43
10% Lignin with 15% Sorbitol	
Sample	Bendtsen roughness (mL/min)
LS10 A	147 ± 40
	183 ± 147
LS10 B	118 ± 75
	129 ± 129
LD10 A	88 ± 50
	42 ± 44
LD10 B	73 ± 34
	104 ± 55

Table 5: Measures for surface smoothness of samples

For a better visualization and comparison between both sides of the film, the following graph was generated from this data:



Graph 1: Comparison of smoothness between upper and lower side of a film.

Most data recorded for the smoothness of samples have an important value of variance associated to the measurement. Data collected shows a difference in smoothness between the sides of a same sample. In some cases, this difference is within the tolerance established by the measurements, which would imply that values are similar for both sides. In other cases, the values are clearly different depending on the side of the film measured. This data shows that the variance for this property was very significant, which could imply that the films were not as homogenous as expected.

3.2 Barrier properties

Barrier properties measure how a sample can protect one side from the effects on the other side. These properties should not be affected by the side of the film being tested, as the agent being tested must go through both sides of the film. The properties considered vital in the testing of a film were: how it reacted with water, and with air. From other tests already developed cellulose films are completely resistant to oils and fats (Tyagi et al. 2019). Therefore, this barrier property was not tested or evaluated in this study, since this is a proven property.

Testing how film samples behaved under the effect of water was an essential reason why lignin was added to nanocellulose. To properly evaluate the effects of lignin and other additives on the sample's ability to repel water, or hydrophobicity, three different tests were evaluated.

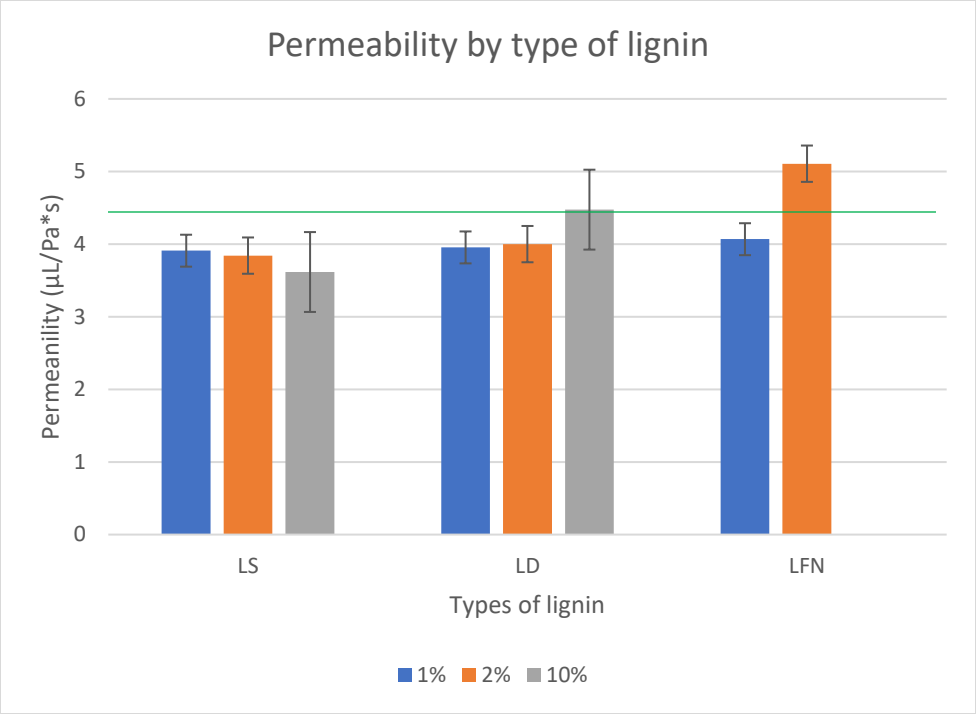
3.2.1 Air permeability

The following table shows the values for air permeability measured in the Celbiotech laboratory. For these measurements, one sample of each composition was used.

Addition of 15% Sorbitol		
Sample	Permeability (mL/min)	Permeability (µm/Pa*s)
C C	390	4.41 ± 0.20
CN B	404	4.57 ± 0.42
1% Lignin with 15% Sorbitol		
Sample	Permeability (mL/min)	µm/Pa*s
LF1 B	324	3.66 ± 0.23
LFN1 A	360	4.07 ± 0.22
LS1 A	346	3.91± 0.17
LSU1 A	340	3.84 ± 0.23
LD1 A	350	3.96 ± 0.28
2% Lignin with 15% Sorbitol		
Sample	Permeability (mL/min)	µm/Pa*s
LS2 B	340	3.84 ± 0.13
LD2 B	354	4.00 ± 0.23
LFN2 B	452	5.11 ± 0.37
10% Lignin with 15% Sorbitol		
Sample	Permeability (mL/min)	µm/Pa*s
LS10 B	320	3.62 ± 0.38
LD10 B	396	4.47 ± 0.70

Table 6: Values for air permeability of samples errors.

Values for the first batch of samples, containing a 1% concentration of lignin show a reduction in the value of air permeability in comparison to the control sample, C C. This can be observed by the horizontal green line that appears in Graph 2 to indicate the value of this C sample. With a higher increase in the concentration of lignin, in the 2% range, permeability does not vary for LS and LD, but it does vary with the addition of NaOH. The effect is noticeable for the sample containing only NaOH but is even greater with the addition of flax lignin. This type of lignin had the lowest value for air permeability with lower concentrations, of 1%. In the last batch of testing, with both LS and LD, a trend can be observed where addition of LS decreases the value of air permeability. Instead higher concentrations of LD increase the values for this barrier property.



Graph 2:Permeability by type of lignin and concentration, green line indicates control.

To put this data into context, a porous filter papers have values of permeability ranging between 7.4-52.9 µm/Pa*s (Fillat et al., 2018). Therefore, in comparison to this material, the permeability of samples generated is lower in all cases, even considering the lowest values of this range. For certain applications having low permeability may be an essential characteristic.

3.2.2 Water drop test

Water drop tests were conducted throughout this study in order to evaluate if additives to CNC films had any effect on the hydrophobicity and capacity of absorption of sample films. The following table show the values resulting from experimentation:

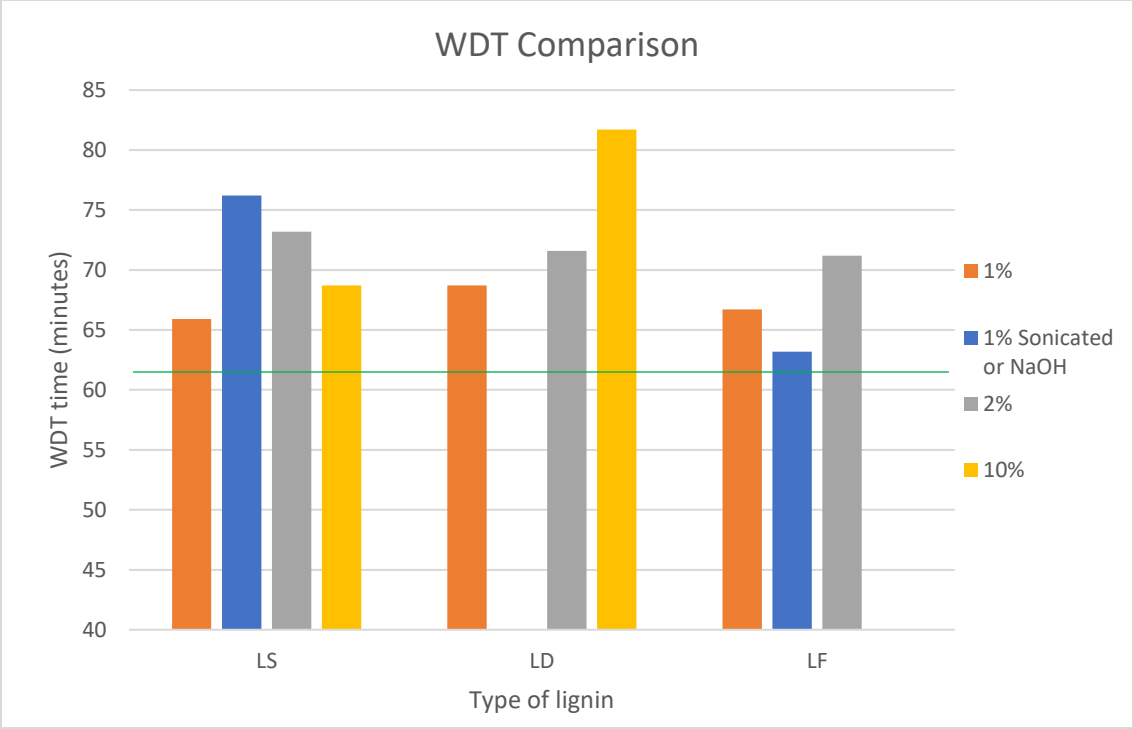
Sample	Time (min)
TC	20.1 ± 0.2
TLF1	43.1 ± 5.5
Addition of 15% Sorbitol	
Sample	Time (min)
C	62.2 ± 2.2
CN	59 ± 7.5

1% Lignin with 15% Sorbitol	
LF1	66.7± 3.2
LFN1	63.2 ± 4.4
LS1	65.9 ± 4.5
LSU1	76.2 ± 0.5
LD1	68.7 ± 4.8
2% Lignin with 15% Sorbitol	
Sample	Time (min)
LS2	73.2 ± 1.5
LD2	71.6 ± 0.8
LFN2	71.2 ± 7.0
10% Lignin with 15% Sorbitol	
Sample	Time (min)
LS10	68.7 ± 4.7
LD10	81.7 ± 0.8

Table 7: Results for water drop test – Water Absorption

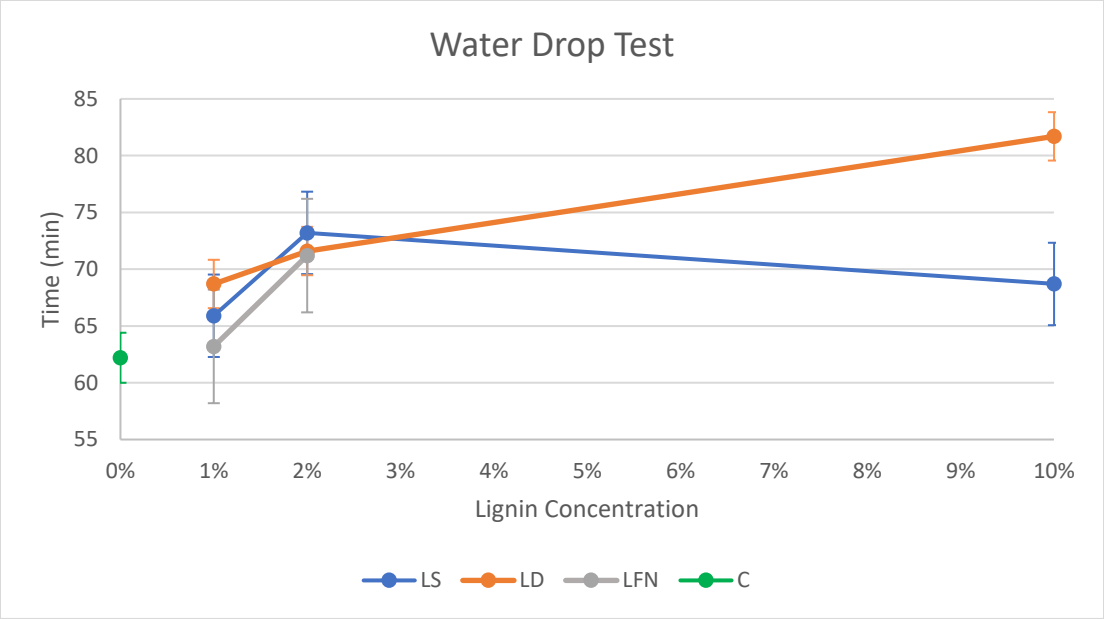
Measurements for the average time in the water drop test indicate an increase of more than 100% due to the addition of lignin in the first samples tested, with 1% concentration of lignin. Other samples with the same concentration of lignin also had sorbitol added as a plasticizer. Once the plasticizer was added, the increase in time was even higher. Control samples with only sorbitol took almost three times as much time to absorb the same drop than a sample without sorbitol. Therefore, the effect this substance had on the sample's ability to absorb water was evident.

With lignin added to the samples, the resulting times are also higher, but only by a 6% increase on average. As Lignin concentration was increased, so was the average time samples took to absorb water. This increase was not as effective as in the initial samples that contained no sorbitol. Hydrophobicity of samples increased when lignin was added, but this effect was masked by the stronger effect of sorbitol. In graph 3, different types of lignin are compared with the control sample which is shown with a green line.



Graph 3: WDT results for different lignin and concentrations, green line indicates C.

LSD10, a sample containing 10% concentration of LD and 15% concentration of sorbitol obtained an average time of 81.7 minutes. In comparison with the same sample with a lower concentration of lignin, results show 13 minutes of absorption time were added due to an increase of ten times the concentration of lignin. In these same conditions, the LS sample shows an increase in the time, but in a far less significant manner. For this type of lignin, values of 2% concentration show the highest resulting absorption time. This suggests that this property is saturated, or that the addition of more lignin will not necessarily increase with a higher concentration. LS at such high concentrations perhaps precipitated once it was placed in the mold, which could mean that this substance was not fully dissolved in the sample.



Graph 4: Water drop test time with different concentrations of lignin.

Data from the water drop test shows that adding lignin to a sample results in an increase in the time this samples takes to absorb a drop of water. This effect is less pronounced than the effect of adding sorbitol, which was initially added to facilitate handling the sample. Sodium hydroxide seems to have a slightly negative effect on the samples ability to absorb water as can be seen by comparing the same samples with and without this additive.

Addition of 15% Sorbitol			
Sample	Time (min)	Sample	Time (min)
C	62.2 ± 2.2	CN	59 ± 7.4
LF1	66.7 ± 3.2	LFN1	63.2 ± 4.5

Table 8: Comparison between samples with and without sodium hydroxide.

This substance allows for LF to dissolve in CNC solution, generating an increase in hydrophobicity overall, since the lignin is more effective than the contrary sodium hydroxide effect. Sodium hydroxide dissolves easily in water, which shows that the effect of NaOH is still active in the films. Sodium hydroxide also has a structural effect over the composition of films, which could affect their ability to absorb water. Visually, films that are composed with the use of sodium hydroxide show different patterns, showing that this substance has an effect over the disposition of CNC crystals. A visual reference of this effect can be seen in Table 2, which

shows all sample films and the pattern created in sodium hydroxide samples (contain N in nomenclature). In fact, studies show that NaOH has an effect on the distribution of nanocrystals during film formation (Parit et al. 2018).

This data also shows a 15% increase in the LS samples simply by sonicating the lignin before adding it to the solution. LSU1 sample was generated by applying ultrasound sonication, explained in section "2.2.1". Further testing is necessary to determine the full extent of this effect, but sonication allowed for a more homogenous solution, which increased the water drop test time for the same type of lignin.

3.2.3 Contact angle

The following table shows values for the contact angle between the indicated sample and a 4µl drop of water. This angle is an indicator for the samples' hydrophobicity.

Addition of 15% Sorbitol		
Sample	Contact Angle (degrees)	
	Left Angle	Right Angle
C	49.9	51.4
CN	45.2	44.6
LF1	54.3	54.3
LFN1	40.8	41.4
LS1	63.5	63.8
LSU1	64.4	64.4
LD1	57.0	57.3
2% Lignin with 15% Sorbitol		
Sample	Contact Angle (degrees)	
	Left Angle	Right Angle
LS2	46.3	46.9
LD2	48.6	48.7
LFN2	49.8	50.3
10% Lignin with 15% Sorbitol		
Sample	Contact Angle (degrees)	
	Left Angle	Right Angle
LS10	59.3	59.4
LD10	52.3	52.1

Table 9: Contact angle between water drop and sample.

Data for the contact angle between a water drop and samples does not indicate any clear trend. Hydrophobic substances show values for contact angle greater than 90º, angles above 130º are considered very hydrophobic. Samples tested all fell within the hydrophilic range of contact angles. However, they were higher than typical papers that are within the range of 24 and 33º (Fillat et al., 2018). An increase in the concentration of lignin was expected, due to WDT results, to increase the contact angles. Instead, results showed no real trend, generally decreasing in the 2% concentration range and then increasing for 10% concentrations. A higher concentration of lignin can cause samples to not be fully dissolved, which affects the smoothness of the surface and therefore can generate changes in the contact angle.

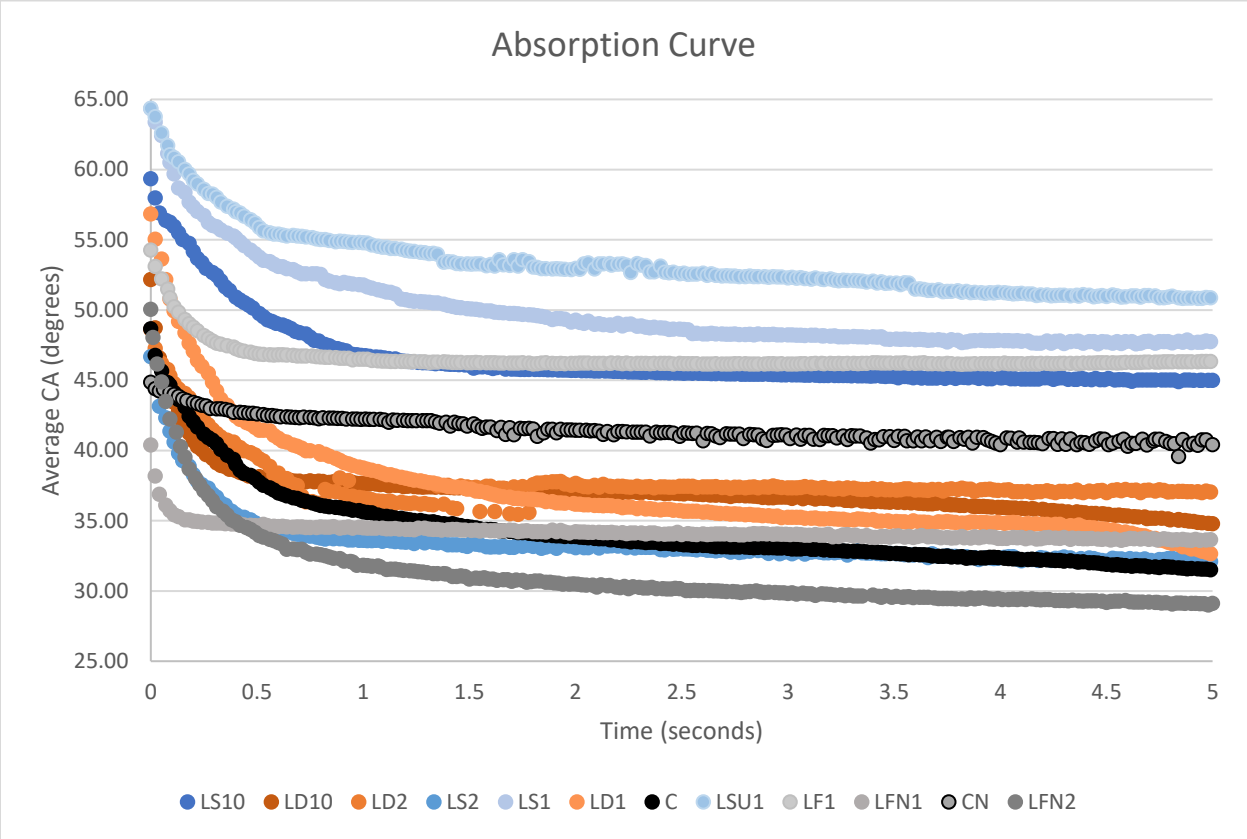
A similar effect to the WDT test can be observed in two different aspects. First, sodium hydroxide samples tend to reduce their contact angles, implying that there are less hydrophobic when the substance is added. The samples compared have the same CNC, sorbitol and LF concentrations, the only change is the addition of NaOH. The second case in which this test is similar to the WDT results is the increase in angle between the LS samples that have been ultrasonically mixed and those that have not. LSU1 sample shows the highest value for contact angle.

Comparing the different types of lignin added to the samples, LS performs best. This lignin has the highest value of contact angle except for the 2% concentration range, where values are all in a very similar range. This type of lignin dissociates in water; therefore it is also the one that is best dissolved in the solution, forming the most homogenous sample.

3.2.4 Absorption curve

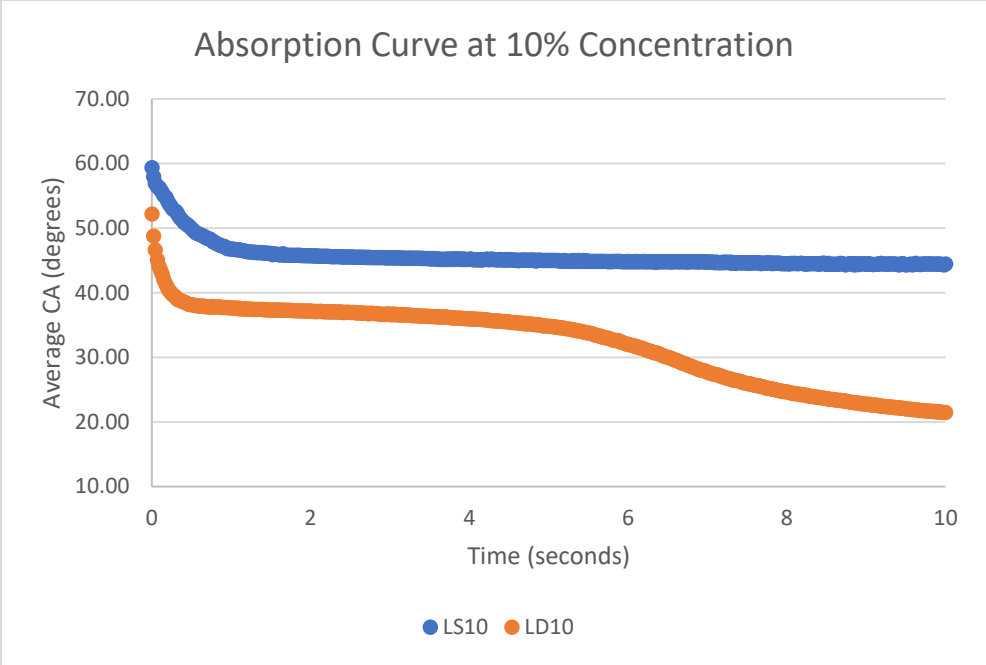
Absorption curve allows to measure how the contact angle between the sample and a single water drop changes over time. To analyze this data, it was extrapolated in different graphs. Various sets of data were measured for each sample, graphs were generated with averages of sets of data. In some cases, measurements present anomalies due to the film moving once the drop entered in contact with the sample. This effect changed parameters set by the software and changed values for some readings. These data sets were not used for the following results.

Data is organized by colors to show samples depending on the type of lignin used in their composition, and darker colors show higher concentrations. All CNC and sorbitol samples show a similar behaviour in the first second since they enter contact with the water drop. The reduction of contact angle in the first second after impact follows and exponential function. This implies that the contact angle reduces quickly and then tends to stabilize during a few seconds. Graph 5 was generated with the intention to show all data together, allowing to visualize that darker colors do not imply a higher value for the contact angle. In fact, for all types of lignin, the highest curve does not belong to the sample with the highest value for lignin concentration.



Graph 5: Absorption curve for all samples tested.

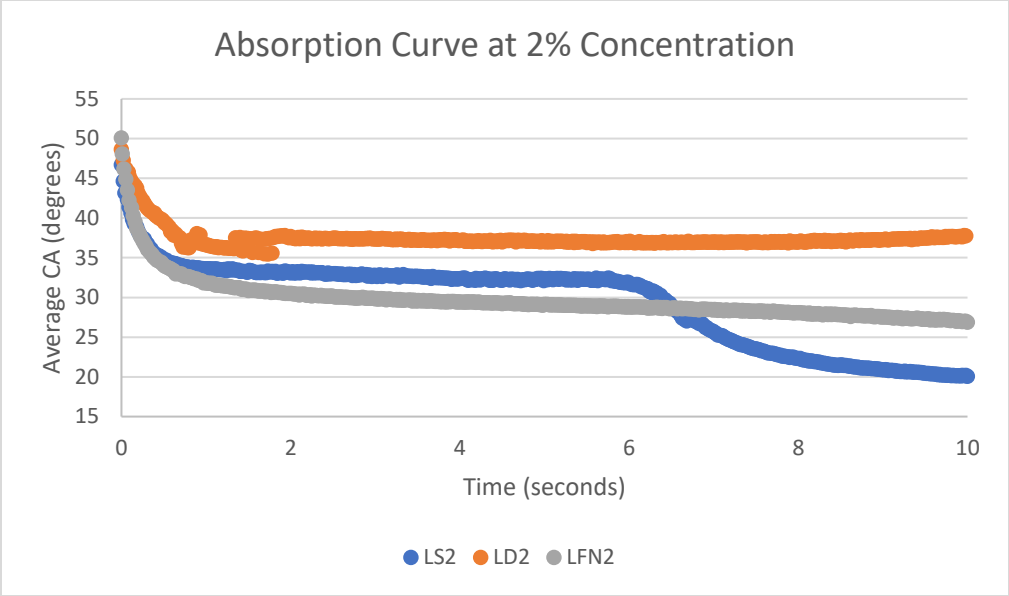
In order to analyze the differences between types of lignin added to CNC samples at the same concentrations the following graphs were created. To begin with the curves for lignin at a 10% concentration will be analyzed.



Graph 6: Absorption curve for LS and LD at 10% concentration

Data for 10% concentration of lignin shows that LS had a higher starting point, the contact angle at 59.3º, but was stabilizing more effectively than the LD sample. The LD sample seems to stabilize around the 37º value, but then has another drop and stabilizes again at around 20º. This effect was observed in a variety of samples, where the sample experienced an initial drop and a second less pronounced reduction in the CA. LS values for WDT were less than those measured by LD, which is contrary to what is expected with CA and absorption curve data collected. This data indicates that LS is better at repelling water initially but throughout longer period of time, LD is more hydrophobic. Both curves were higher than the control curve, which implies that lignin does have a positive effect in making samples less water absorbent.

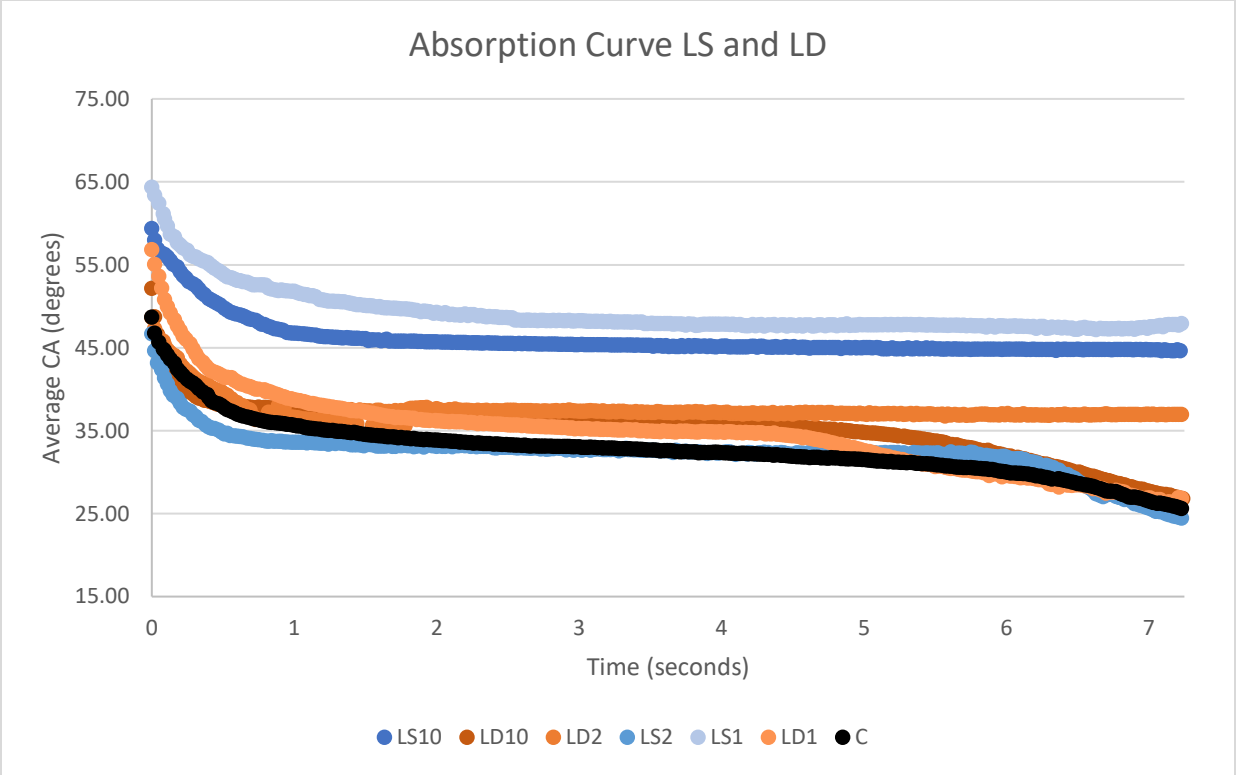
To further analyze these differences different concentrations will be evaluated adding LF to allow for further comparison. In graph 7 the three types of lignin are compared at a 2% concentration. This graph shows the same effect than in graph 5, but this type for LS. The sample has a rapid reduction in contact angle and then stabilizes. After 6 seconds, it starts to reduce its' value until it eventually stabilizes again at around 10 seconds. Instead, the CA for LD and LF seems to be stable throughout this period. Data collected for the CA in the previous section "3.2.3", shows LF presents the highest value within the range of this concentration. Instead when this angle is measured over time, it experiences a rapid reduction, with a more pronounced slope than the other lignin types.



Graph 7: Absorption curve for LS, LD and LF at 2% concentration

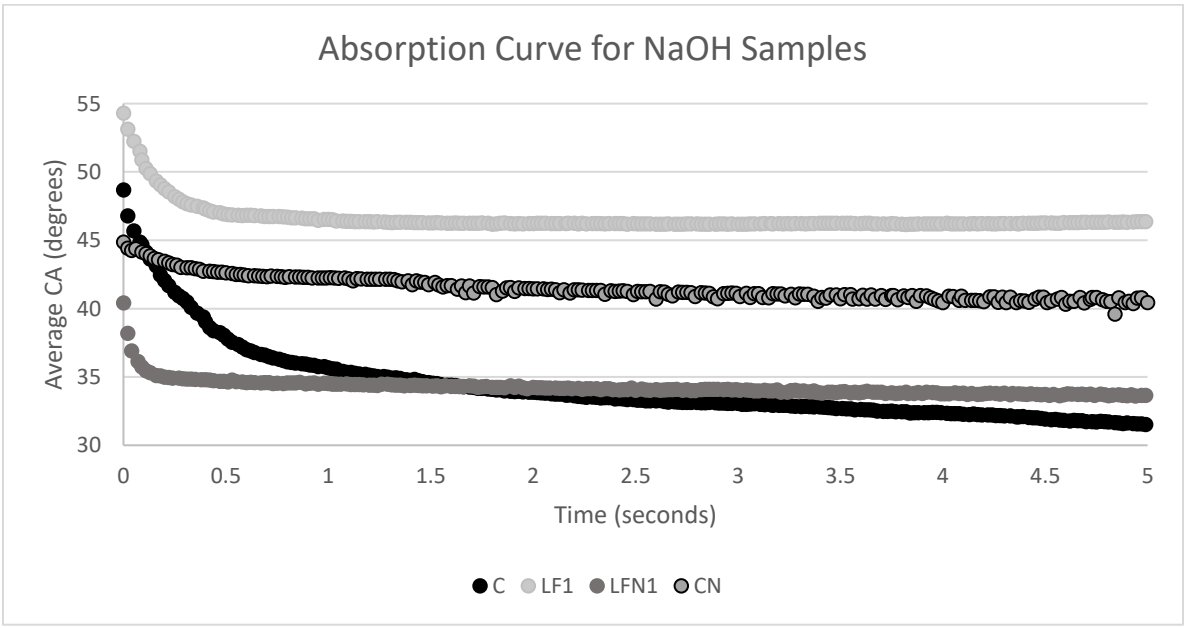
From data analyzed a clear relationship between absorption curve and the concentration of lignin in a sample cannot be established. The following graph places all LD and LS samples together in comparison to the control sample. Samples that had other additives or were generated with other production methods were not added. The reason for this is that the intention of this graph is to analyze the effect of different levels of concentrations on this property, without any other variables to consider.

Graph 8 shows LS and LD samples with different colors depending on their concentration, the same way this data was presented in Graph 3. Data on this graph shows how adding lignin to a sample will generally offer higher hydrophobic properties. A few samples; LS2, LD10 and LD1 have very similar values to the control sample but offer higher results in WDT and are above C in some sections of the graph.



Graph 8: Absorption curve for LS and LD at different concentrations.

Throughout hydrophobicity tests, the addition of sodium hydroxide to samples has reduced the values of the variables used to determine this property. The following graph compares samples with sodium hydroxide and their same composition without this compound.



Graph 9: Absorption Curve for samples with sodium hydroxide

Control samples with sodium hydroxide show a greater value for the absorption curves. Even the initial drop for the sodium hydroxide control sample, CN, is less steep than for all other samples and stabilizes after a 1 second period. C sample still has a negative slope after the initial steep decrease and doesn't seem to stabilize within this period. This data shows that adding sodium hydroxide to a control samples, results in a greater absorption curve. The CA value is greater for the sample without NaOH, but the decrease is a lot steeper in the first second of contact. With LF added to the composition of the sample, having NaOH implies it has a decrease in the absorption curve. LFN1 also stabilizes in a rapid manner, implying that NaOH reduces the initial effect of the contact between water and sample, but does not necessarily make the sample more hydrophobic.

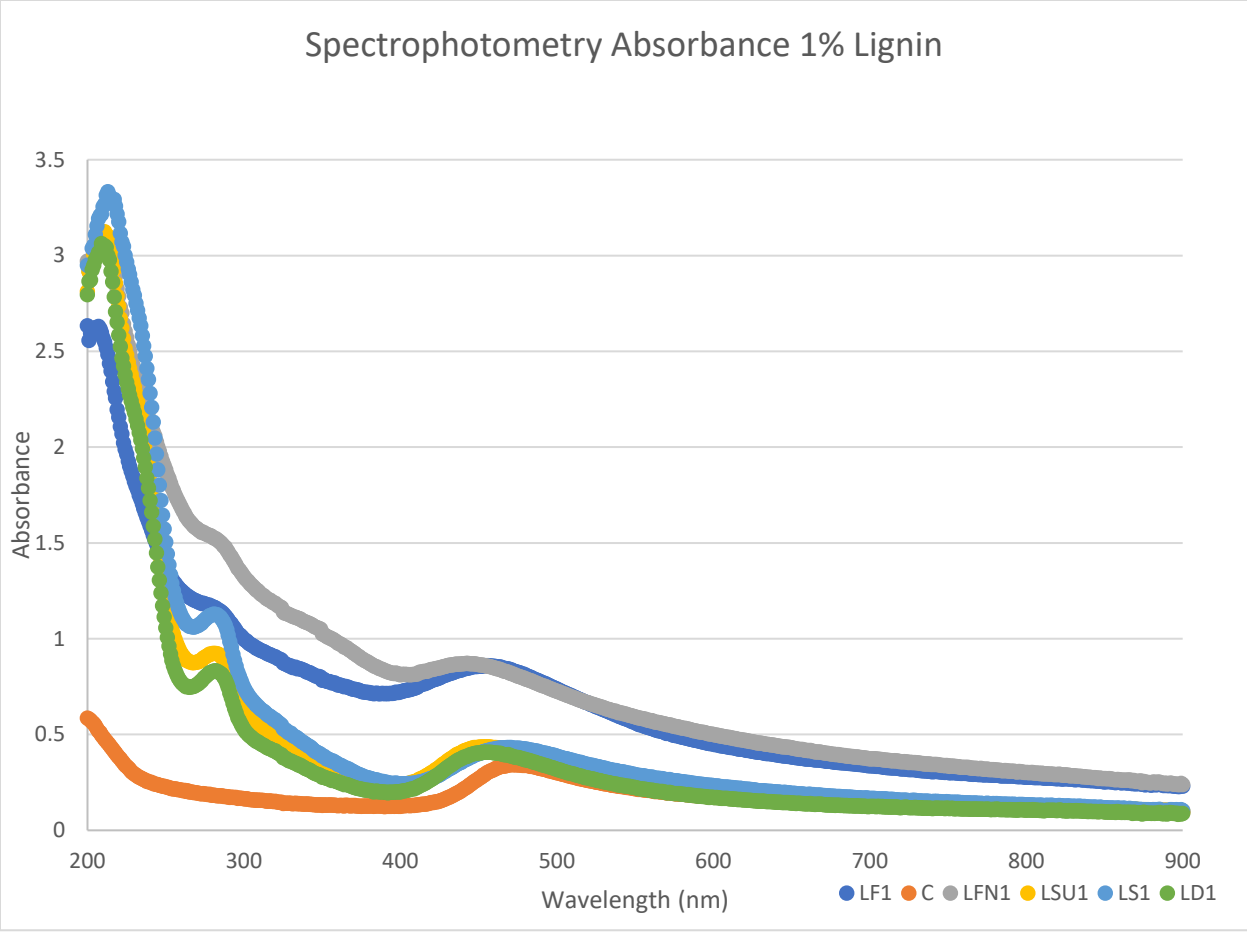
3.3 Optical properties

Optical properties measured in this section are important in a variety of functions. Throughout this section absorbance, specular gloss, transmittance and transparency will be evaluated and analyzed for the different composition tested.

3.3.1 Absorbance

Absorbance values were evaluated in the range of 200-900nm to analyze the effect of both visible light and UV light, both rays generated by the sun. Throughout this section it is important to consider UV light is separated in three different subtypes, as explained in section "1.5.3".

The study of absorbance for a film will determine if a sample is better at protecting a product from specific wavelengths of the light spectrum. To analyze this effect, a variety of graphs were assembled. The first graph shows the effect of adding a 1% concentration of lignin to the samples in comparison with a C sample which is made of only CNC and Sorbitol.

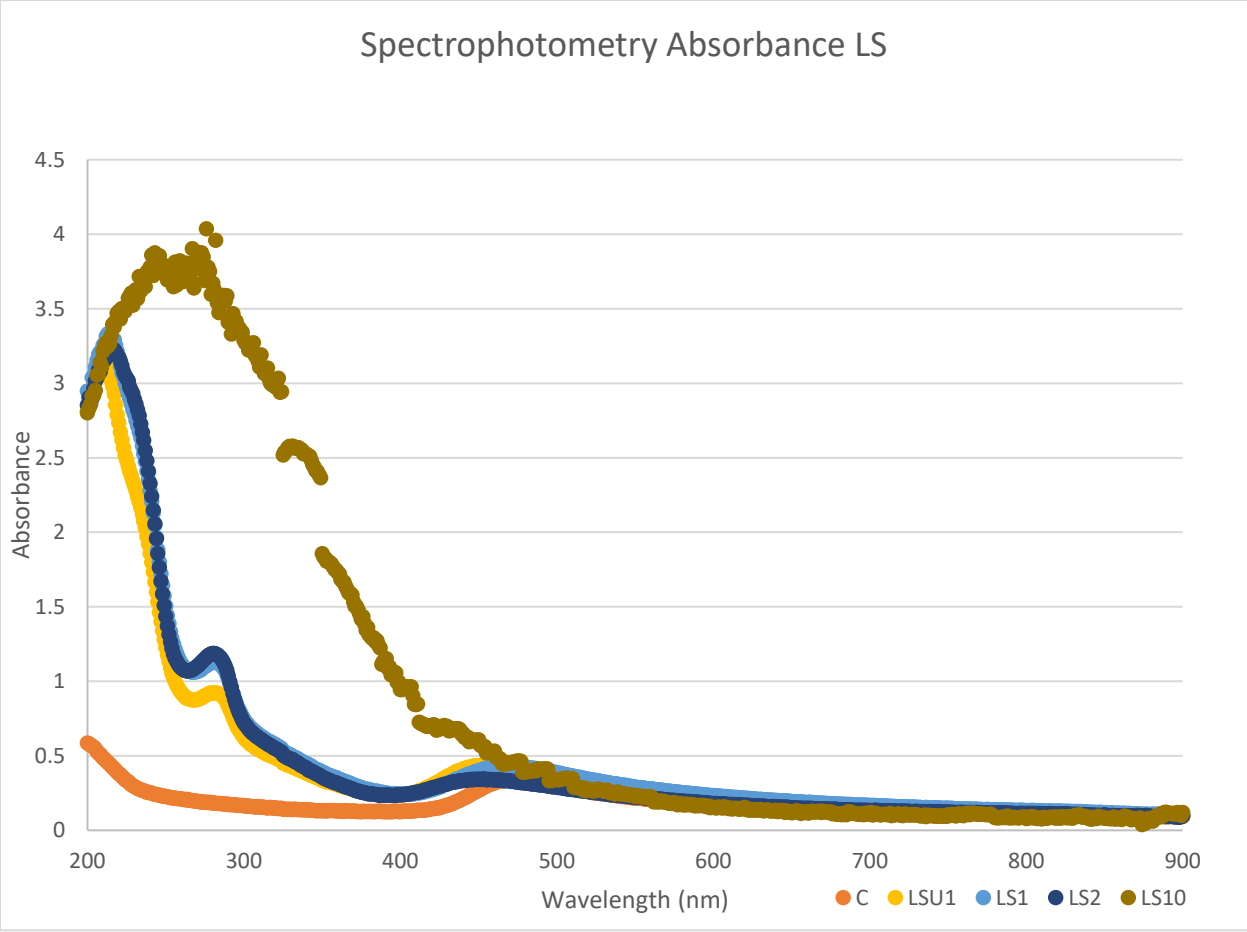


Graph 10: Absorbance curve for 1% concentration of lignin

Graph 10 shows how absorbance values vary with the change in wavelength being emitted to each sample. All samples with lignin added to them show a far greater value for absorbance. Both LF samples show a greater absorbance throughout the whole spectrum, while other types of lignin have a tendency of getting closer to the values of the C sample.

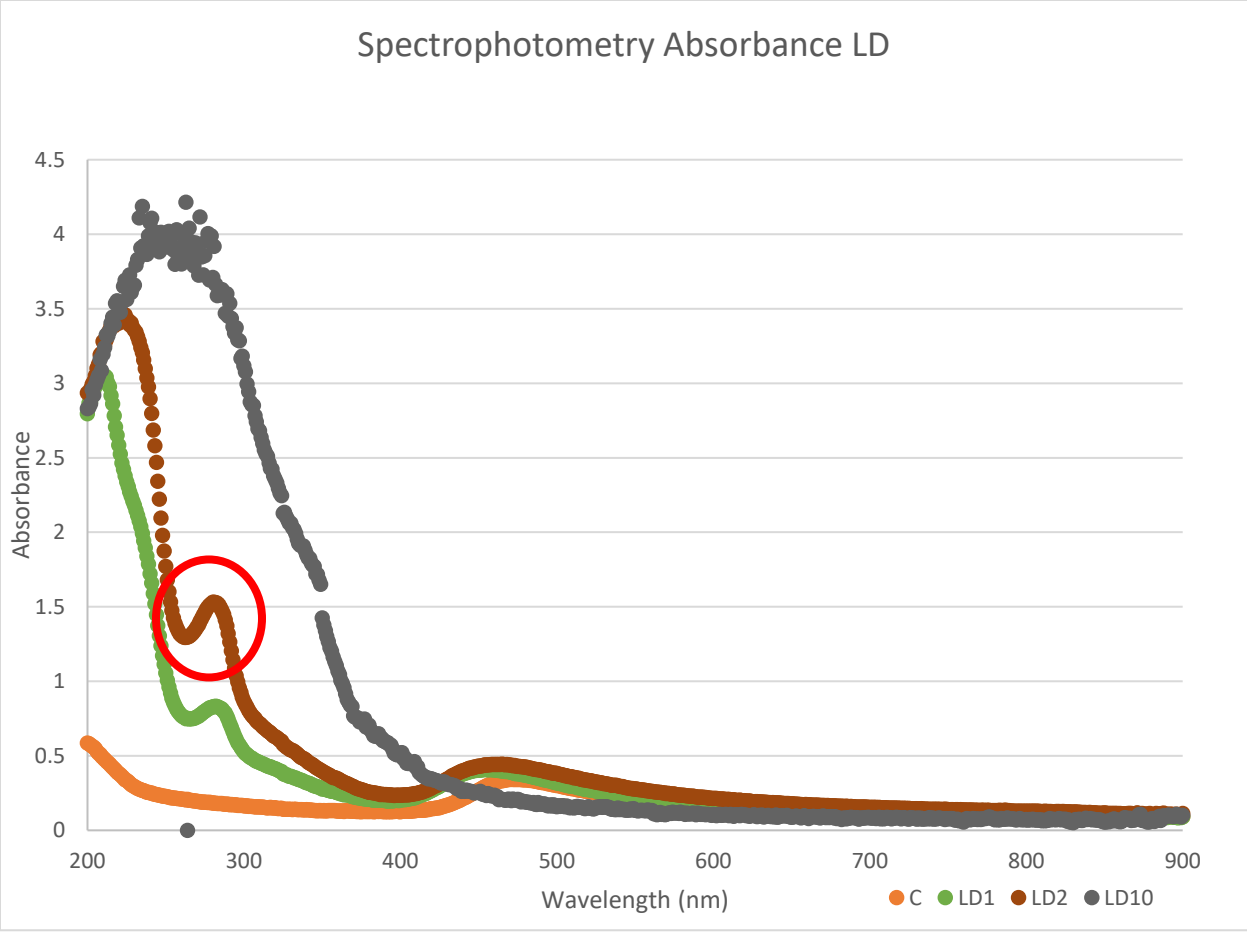
Absorbance values between the 200-500nm range of the spectrum show the effect of adding lignin to a film. All samples within this range of the spectrum show higher values than the C sample, which means they are better at absorbing light. Lignin is light ray absorbent between 280-300nm, this is the reason why in that section of Graph 10, a new curve is generated. UV light corresponds to the initial section of the spectrum, up to 400nm. Lignin samples all have peak absorbance values higher than 2.5, about 5 times greater than the highest value for C samples. These results show that lignin is a very effective polymer to protect from UV rays.

The following graph is aimed at comparing the effect of lignin concentration on the values of absorbance to evaluate if higher lignin concentrations allow for better light protection.



Graph 11: Absorbance of LS in response to different wavelengths.

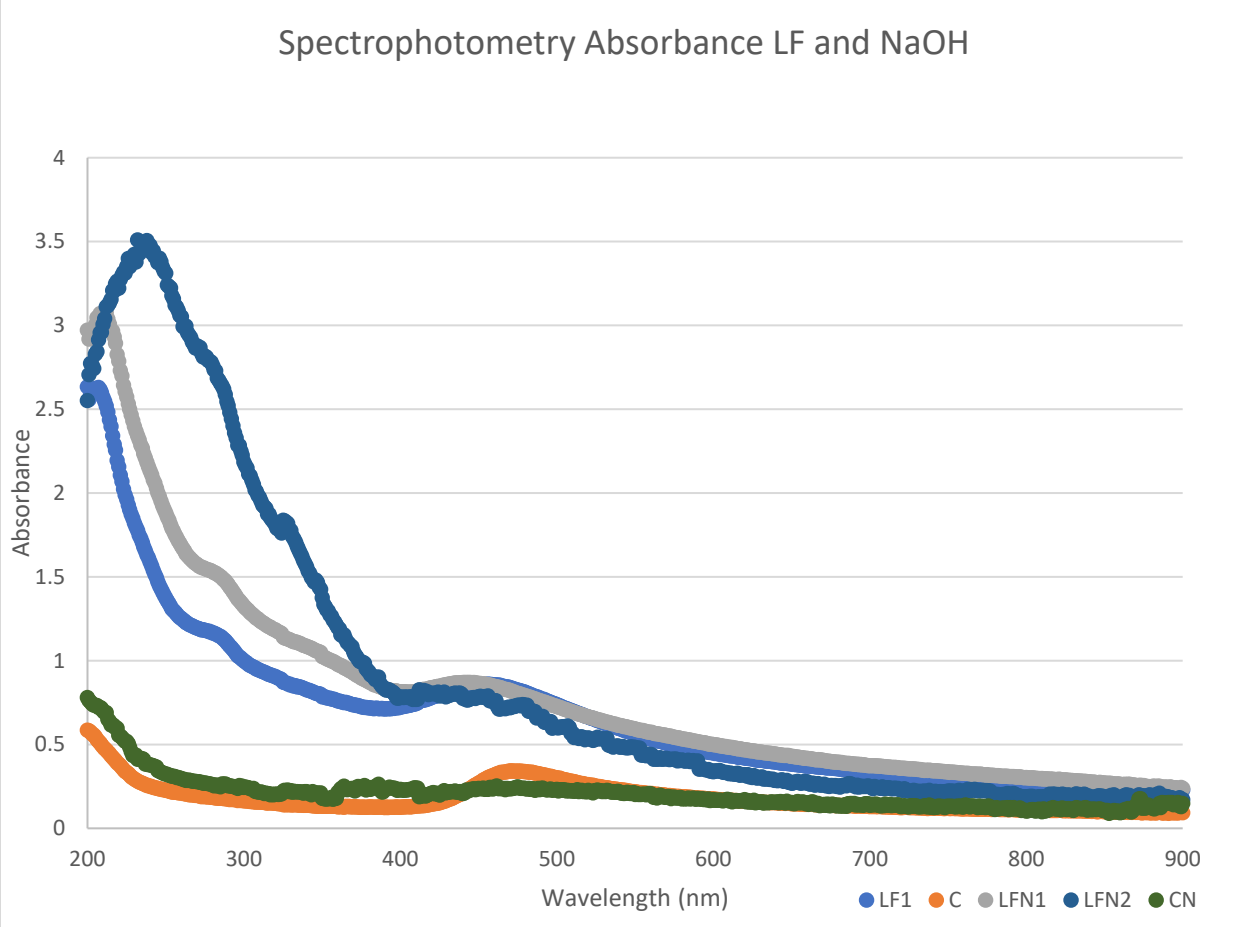
Absorbance values in Graph 11 before wavelengths reach 500 nm, show that in that section of the spectrum, higher lignin concentrations allow for higher absorbance values. In the analysis of LS, LSU1 values show the least peak and general values for absorbance. This sample was mixed with the use of an ultrasonic agitator, resulting in a more homogenous sample. The two blue curves correspond to LS1 and LS2, the difference in a 1% concentration increase is not pronounced. Once the value of lignin increases up to a 10% concentration, for sample LS10 the peak value is reaching an absorbance of 4. This data shows how an increase in the concentration of lignin is directly proportional to an increase in the sample's absorbance. The presence of lignin can be identified by the curve generated at around 280 nm. In the LS10 sample, this curve seems to merge with the initial peak value, generating above 1 absorbance values throughout the whole UV spectrum of light.



Graph 12: Absorbance of LD in response to different wavelengths.

Values of peak absorbance in Graph 12 are even higher than in Graph 11. This data, once again, shows how an increase in the concentration in lignin generates higher values in the absorbance between 200 and 400 nm. The curve generated by lignin at around 280 nm is circled in red (Parit et al. 2018). It is more pronounced as the concentration increases. Like in LS samples, once the concentration is at 10%, the curve merges with the initial peak. After the UV section of the light spectrum, samples have the same values of absorbance for light in the visible spectrum.

Initially at 1% concentration values, LF showed the highest values for absorbance. In order to evaluate this and the effect of NaOH on the absorbance of films the following graph was assembled.



Graph 13: Absorbance of LF and NaOH samples

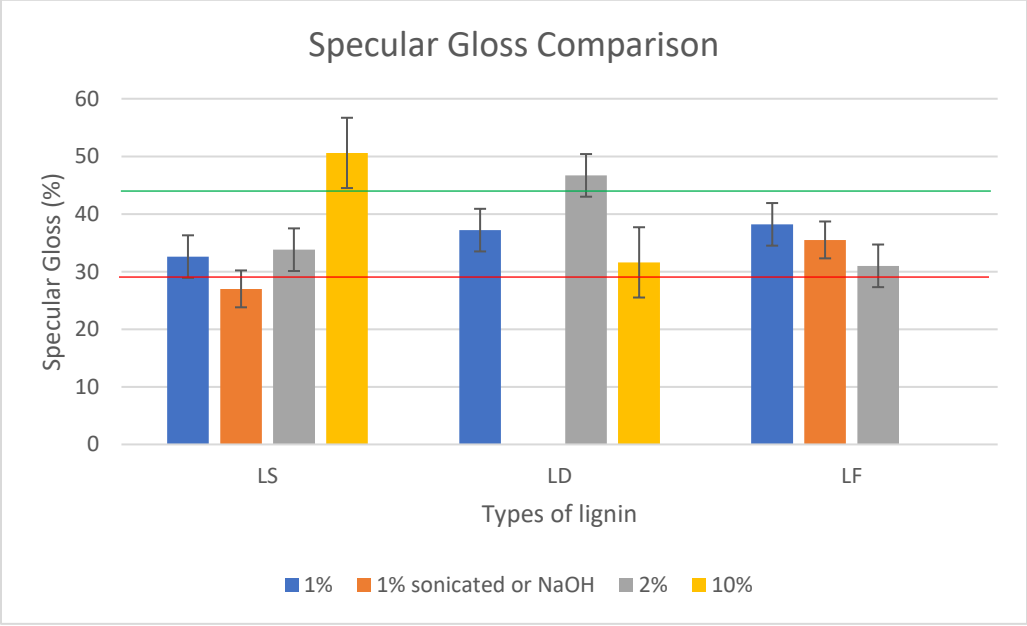
LF samples show greater peak values for absorbance than other lignin types for the same concentrations. Instead, the second curve around 280-300nm is a lot less pronounced in these samples. LF also has higher values of absorbance throughout a longer range of the spectrum, consistently showing better absorbance values than both control samples. Sodium hydroxide added to the samples presents an increase in absorbance which is more effective when LF is added. For the same concentration of LF, at 1%, the sample with NaOH, LFN1 shows higher absorbance values. This data shows that the addition of NaOH to samples does not harm their ability to absorb light. In fact the addition of NaOH to samples allows for LF to dissolve easily, which allows for it to be more effective in absorbing light. This difference can be appreciated in samples LFN1 in comparison with sample LF1, which contain the same amount of lignin but the addition of sodium hyrdoxide has allowed for it to dissolve better in throughout the film. This generates a more homogenous film that is better at absorbing light energy.

3.3.2 Specular gloss

Specular gloss tries to measure how a specific sample is capable of reflecting light. Values for the specular gloss of samples created are presented in the following table and graph:

Addition of 15% Sorbitol	
Sample	Specular gloss (%)
C	43.7 ± 2.6
CN	29.3 ± 5.9
LF1	38.2 ± 2.7
LFN1	35.5 ± 3.1
LS1	32.6 ± 5.1
LSU1	27.0 ± 3.3
LD1	37.2 ± 3.3
2% Lignin with 15% Sorbitol	
Sample	Specular gloss (%)
LS2	33.8 ± 4.6
LD2	46.7 ± 1.5
LFN2	31.0 ± 5.9
10% Lignin with 15% Sorbitol	
Sample	Specular gloss (%)
LS10	50.6 ± 8.9
LD10	31.6 ± 1.3

Table 10: Specular gloss with incident light ray at 20º



Graph 14: Specular Gloss for different types of lignin and concentration, Green-C and Red-CN.

Data collected from measurements for specular gloss show many different responses for this property. Initially with lignin at a 1% concentration, the addition of lignin reduces the specular gloss of a CNC film. All values for specular gloss in samples with 1% concentration are less than the control sample, C. Control sample is shown in the graph with a green line, while the control sample value for NaOH, CN is shown in red. Visually, Table 2 for reference, CNC samples had an iridescent visual effect that was diluted by the addition of lignin. For an even higher concentration of lignin at 2%, this effect follows a similar trend, except for LD2 sample which has an increase in the value of specular gloss. With concentrations of 10% lignin, LS10 values increase, while LD10 decrease. The addition of lignin doesn't imply a reduction in the specular gloss of a sample. For low concentrations of lignin, a tendency to reduce the specular gloss exists, but this is not true for any concentration.

Sodium hydroxide does have a clear effect on the specular gloss of films. This can be seen visually, as light reflects in different patterns when NaOH is added to a sample. Specular gloss allows for this effect to be quantified.

Addition of 15% Sorbitol	
Sample	Specular gloss ()
C	43.7 ± 2.6
CN	29.3 ± 5.9
LF1	38.2 ± 2.7
LFN1	35.5 ± 3.1

Table 11: Effect of NaOH on specular gloss of a sample.

Values for specular gloss with NaOH added to the sample are reduced. This effect is masked when lignin is added to the sample. With control samples, an addition of NaOH generated an 26% reduction in the value of specular gloss.

3.3.3 Transmittance

Values for transmittance were measured with the spectrophotometer setting the variable for wavelength at 600 nm. the following data was collected:

Addition of 15% Sorbitol	
Sample	Transmittance (%)
C	66.21 ± 0.03
CN	69.13 ± 0.01
LF1	40.22 ± 0.01
LFN1	40.50 ± 0.01
LS1	58.22 ± 0.02
LSU1	60.29 ± 0.02
LD1	58.77 ± 0.02
2% Lignin with 15% Sorbitol	
Sample	Transmittance (%)
LS2	66.06 ± 0.01
LD2	59.60 ± 0.01
LFN2	47.89 ± 0.01
10% Lignin with 15% Sorbitol	
Sample	Transmittance (%)
LS10	67.94 ± 0.14
LD10	78.83 ± 0.01

Table 12: Values for % transmittance at 600 nm. wavelengths.

Values for transmittance indicate the amount or portion of light that is transmitted through a film. This means that high values of transmittance indicate that a larger portion of incident light is transmitted towards the other side of the film. To a certain extent, transmittance also takes into account values for electromagnetic radiation absorption measured in the previous section. At 600 nm. wavelength this value corresponds with visible light in the electromagnetic spectrum, specifically with orange colored light.

Values of transmittance at this wavelength show an insignificant amount of variance in the measurements. This implies that measurements for this property did not vary a lot throughout the films. Visually, more opaque samples such as those with a higher concentration of lignin are expected to have better results in blocking incident rays of light. This hypothesis is not true since the samples with the highest concentration of lignin, LS10 and LD10, also show the highest percentage of transmittance. In all samples, an increase in the amount of lignin generates an increase in the percentage of light that is transmitted through the film.

Have lignin added to a film does reduce the percentage of light transmitted, as can be observed when comparing samples with 1% concentration of this substance with the control samples. Data shows that once the concentration of lignin is increased the films are not as effective in blocking light. The sample that performs best is LF, which is also the opaquest colored sample within the 1% concentration of lignin samples. Once the concentration is increased, as with LFN samples, the percentage of light transmitted also increases. This data is interesting since an increase in

concentration of lignin involves greater absorption values, but also implies that more light is transmitted through the film. This could be since at higher concentrations, lignin is not dissolved in the films as effectively, generating structural gaps in the samples. Lignin present in the film would still be capable of transforming incident light into internal energy, through absorption but some of this light still scatters through the film.

3.3.4 Transparency

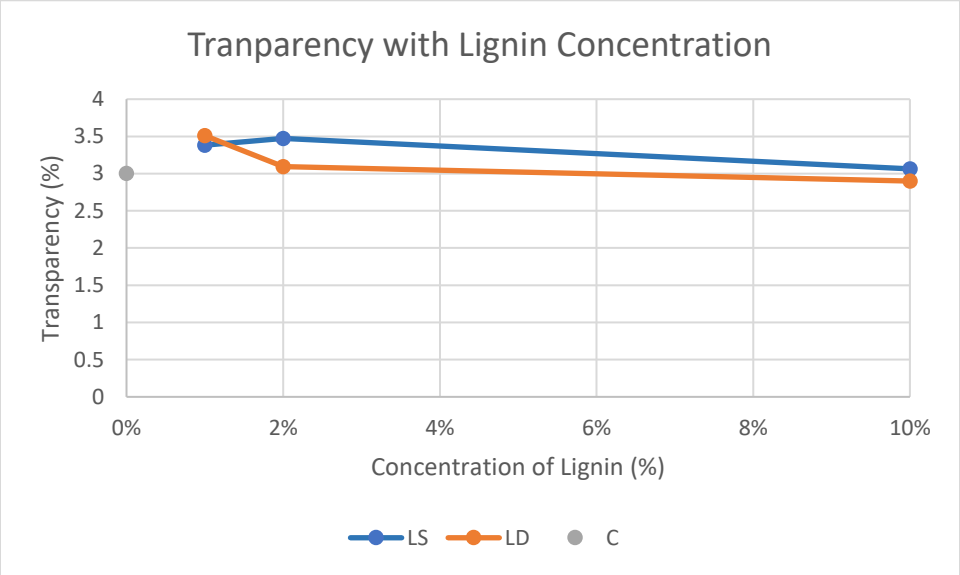
Transparency values were measured both using specific equipment as explained in "section 2" and using a formula. Transparency is a property that can be interpreted visually, and data obtained with equipment was not evaluated since it was not coherent with expected values. Instead, transparency of a film was obtained by calculations from the values of % transmittance at 600 nm. The equation is explained in the calculations annex "section 5" and is a result of a study (Han & Floros, 1997):

Addition of 15% Sorbitol	
Sample	Transparency (%)
C	3.00
CN	4.05
LF1	2.09
LFN1	2.50
LS1	3.38
LSU1	3.23
LD1	3.51
2% Lignin with 15% Sorbitol	
Sample	Transparency (%)
LS2	3.47
LD2	3.09
LFN2	2.58
10% Lignin with 15% Sorbitol	
Sample	Transparency (%)
LS10	3.06
LD10	2.90

Table 13: Values for transparency of a film.

Data obtained for the transparency of films shows that in general terms, looking at the percentage difference, samples do not vary a lot. Variance for this data was so low, since it is obtained from values of transmittance, that it was not added to the table for a clearer interpretation of the data. Transparency values were expected to be higher for control films since

their aspect was less opaque than the samples with lignin added to them. Data shows that with an increase in concentration, transparency is slightly reduced. This effect is hardly noticeable as can be observed in the following graph.



Graph 15: Change in transparency with increase in lignin concentration.

From the data obtained the value of transparency in a film is not strongly dependent on the concentration of lignin in that film. The most significant difference is in samples with sodium hydroxide is added to the film, in all cases in which NaOH was added to the film, values for transparency increased. This could be related to the visual patterns that this additive generates on the surface of a film, or even to the PH of the sample. In general, the control sample has values within the range of samples with lignin. Visually, results transparency values were expected to be far higher for this sample.

4. Conclusion

This study is aimed at the development of a nanocomposite in the form of a film to generate an alternative to petroleum-based plastics. To generate this film, an essential condition was to keep in mind the value of the resources being used, a key concept in biorefineries. In many industries, byproducts are used in ineffective ways or simply considered waste, generating contamination problems in water and air. This study hoped to use one of these byproducts, lignin, to provide properties that would make a completely biodegradable film an alternative to other plastics. Throughout this process, an in-depth analysis of physical, barrier and optical properties have allowed to evaluate the effect of different types of lignin at different concentrations, as well as other additives, on these properties.

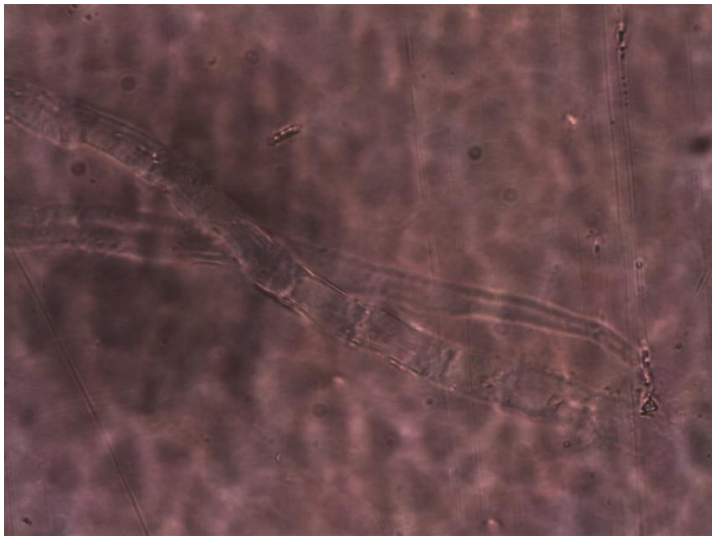


Figure 17: Image of LSFN2 sample at 200x magnification, LF fiber visible.

This study has evaluated a variety of properties that are essential in the function of a film. Physical properties served as parameters to analyze if the addition of lignin to a film had effects on the thickness, grammage and surface smoothness of a film. These physical conditions have an effect on other properties as they show the state of matter in the films. In the study of these properties, values tend to have very significant variance. This fact is also interesting because it shows that the production of films implied that these films were not perfectly homogenous, and data would be affected by the position or specific film used. This was important information to obtain in the beginning of the testing phase because from this point on, data was extracted from the same sample whenever it was possible. By taking this into account the intention was to focus on the concentration of lignin in that sample and not in the irregularities that any sample could have. These measurements were also for relatively small units, such as micrometers, which tend to

have more significant variance levels. In this stage of testing, another effect that could be seen in the film production is the change in smoothness depending on the side of the film tested. The side that was in contact with the mold had a much smoother finish than the upper side of films. This is interesting since if one day a product like this were to be produced industrially, the value for surface smoothness could be altered by the production method.

Barrier properties and optical properties were used to analyze the functionality of film in the scenario that it would be used commercially. The main aspects that were analyzed within the barrier properties of a film were how permeable the films were and how hydrophobic they could be depending on the concentration of lignin added. From the data obtained, significant increases in hydrophobicity were obtained with the addition of the plasticizer sorbitol. Values in the water drop test also increased with an increase in the concentration of lignin, were masked by the effect of sorbitol. Adding lignin to samples makes them more resistant to water, even if these results were not as drastic as expected due to the nature of lignin. Results for the permeability of samples were not significant when it comes to analyzing the effect of lignin to the samples, but they did show values that show CNC films are far less permeable to air than porous paper.

Films with lignin were expected to be capable of giving optical properties that were very interesting in protecting and conserving any material from UV light radiation. Perhaps this property, the ability of absorption of light radiation is the most valuable property added by the addition of lignin to a film. Films with an increase in the concentration of lignin showed a better ability to absorb light radiation, but specifically they showed these values within the range of UV light. The fact that these films were best at protecting from the most harmful band of solar irradiance, means that lignin does add a lot of value to a film. The increase is also extremely significant for this property, films with lignin can be up to 5 times more effective in absorbing light with concentration of 10%, and specifically within the UV range. Other optical properties evaluated a more visual effect of the film. Samples were also observed under a microscope to see if the structural impact of adding lignin to a CNC could be observed.

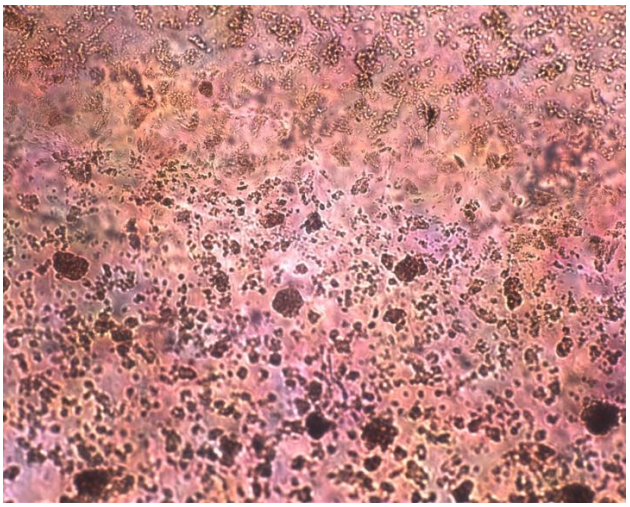


Figure 18: Lignin F at 1% concentration magnified 200x.

CNC films generated an iridescence that was difficult to measure with the properties evaluated. Specular gloss and transparency give an idea of this effect, since they show how light interacts with the surface of film. This effect was changed when the films had NaOH added to them, and slightly disappeared with higher concentrations of lignin. For a visual reference *Table 2* shows the different films produced and their aspect over different bases.

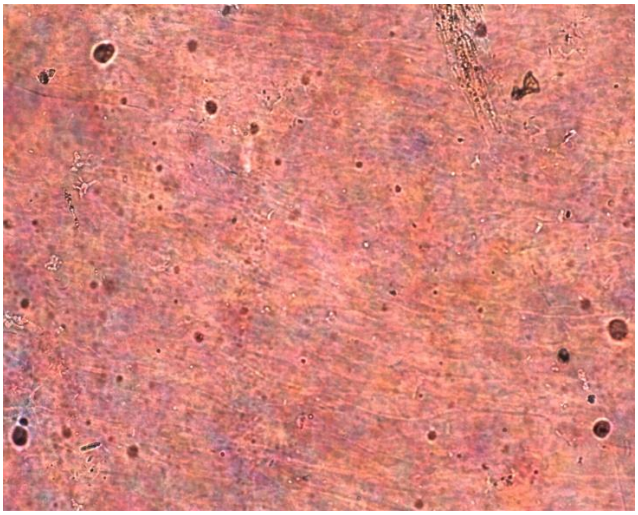


Figure 19: Control sample with sorbitol at a 100x magnification.

This study showed how a variety of properties were affected by the addition of lignin and other additives. The properties selected were those that could have a more significant change with the addition of lignin as well as those that were necessary for the function of a film. Data obtained reflects that adding lignin to a CNC film can be favorable in many aspects, making the film more hydrophobic as well as protective to UV light. Further testing, considering mechanical properties, other types of lignin, more homogenous mixing, and different concentrations would have been interesting. These would be the steps that this study would have followed if time had allowed, due to the promising results in the data obtained up to this point.

5. Calculations Annex

Calculations for Film Production

In order to elaborate films with an approximate thickness of 60 µm, taking into consideration a possible surface area of our mold of 95 cm^2 the amount of solution used was of 40 mL. This data was obtained throughout experimentation by the Celbiotech laboratory.

For a 2% Consistency:

$$1.7g\ CNC\ \times \frac{100g\ total}{2g} = 85g\ Total$$

Grams of CNC solution used at 12.1%:

$$1.7g\ \times \frac{100g\ Sol.}{12.1\ g\ CNC} = 14gCNC\ (at\ 12.1\%)$$

To achieve a 15% concentration of sorbitol as a plasticizer:

$$1.7g\ CNC\ \times \frac{15g\ Sorbitol}{100g\ CNC} = 0.255g\ of\ Sorbitol$$

Lignin, values change depending on concentration desired used (1% Lignin as example):

$$1.7g\ CNC\ \times \frac{1g\ Lignin}{100g\ CNC} = 0.017g\ of\ Lignin$$

To know the grams of distilled water used:

$$85g - 14g\ CNC\ Solution - 0.255g\ Sorbitol - 0.017g = 70.73g\ H_2O$$

➔ The value of distilled water will vary depending on the concentration of lignin used in the sample. This value is approximate, since with the process of drying, the film is dehydrated.

Statistics behind data analysis:

Throughout this study data was collected in sets to find an appropriate way to analyze it. The norm for some properties involved taking various measurements for the same film. The data was treated with statistics to find the best parameters for analysis. To show the steps followed, a specific example of a water drop test is used.

Data collected:

LSS2 Water Drop Test				
Measurement	1	2	3	4
Time (minutes)	75.8	73	72.1	71.9

Average of this data:

$$\boldsymbol{Avg} = \frac{\sum Time}{N.Measurements} = \mathbf{73.2}$$

Standard Deviation:

$$\boldsymbol{\sigma} = \sqrt{\frac{\sum(Avg - Time)^2}{N.Measurements}} = \mathbf{1.56}$$

To take into consideration 95% of the values from the data collected, assuming the data follows a normal distribution:

$$\boldsymbol{Equivalent\ Z - Value\ that\ takes}\ \mathbf{95\%} \rightarrow \boldsymbol{Z} = \mathbf{1.96}$$

Applying formula for confidence interval:

$$\boldsymbol{Z_{0.05}} \times \frac{\boldsymbol{\sigma}}{\sqrt{\boldsymbol{n}}} = \mathbf{1.96} * \frac{\mathbf{1.56}}{\mathbf{2}} \approx \mathbf{1.5}$$

Therefore, for this example:

$$\boldsymbol{LSS2\ WDT\ Value} = \mathbf{73.2 \pm 1.5}$$

Calculations for transparency

In order to calculate the transparency of a film the following formula was used

$$\boldsymbol{Transparency} = \left(\frac{\boldsymbol{log\%T600}}{\boldsymbol{x}}\right)$$

In this formula:

$$x = thickness\ of\ a\ film\ (\mu m)$$

$$\%T600 = value\ of\ transmittance\ at\ 600\ nm.\ (\%)$$

6. Bibliography

Bian, Huiyang, Ying Gao, Zhulan Liu, Weibing Wu, Hongqi Dai, and Ruibin Wang. 2018. "Contribution of Lignin to the Surface Structure and Physical Performance of Cellulose Nanofibrils Film." *Cellulose* 25 (2): 1309–18. https://doi.org/10.1007/s10570-018-1658-x.

Cusola, Oriol, M. Blanca Roncero, Teresa Vidal, and Orlando J. Rojas. 2014. "A Facile and Green Method to Hydrophobize Films of Cellulose Nanofibrils and Silica by Laccase-Mediated Coupling of Nonpolar Colloidal Particles." *ChemSusChem* 7 (10): 2868–78. https://doi.org/10.1002/cssc.201402432.

Ding, Jie, Rong Zhang, Saeed Ahmed, Yaowen Liu, and Wen Qin. 2019. "Effect of Sonication Duration in the Performance of Polyvinyl Alcohol/Chitosan Bilayer Films and Their Effect on Strawberry Preservation." *Molecules (Basel, Switzerland)* 24 (7). https://doi.org/10.3390/molecules24071408.

Feng, Xin, Yafei Zhao, Yaoquan Jiang, Miao Miao, Shaomei Cao, and Jianhui Fang. 2017. "Use of Carbon Dots to Enhance UV-Blocking of Transparent Nanocellulose Films." *Carbohydrate Polymers* 161 (April): 253–60. https://doi.org/10.1016/j.carbpol.2017.01.030.

Han, Jung H., and John D. Floros. 1997. "Casting Antimicrobial Packaging Films and Measuring Their Physical Properties and Antimicrobial Activity." *Journal of Plastic Film and Sheeting* 13 (4): 287–98. https://doi.org/10.1177/875608799701300405.

Klar, Ville, Jaakko Pere, Tuomas Turpeinen, Pyry Kärki, Hannes Orelma, and Petri Kuosmanen. 2019. "Shape Fidelity and Structure of 3D Printed High Consistency Nanocellulose." *Scientific Reports* 9 (1): 1–10. https://doi.org/10.1038/s41598-019-40469-x.

Miranda, Cleidiene S., Marina S. Ferreira, Mariana T. Magalhães, Ana Paula G. Bispo, Jamerson C. Oliveira, Jania B.A. Silva, and Nadia M. José. 2015. "Starch-Based Films Plasticized with Glycerol and Lignin from Piassava Fiber Reinforced with Nanocrystals from Eucalyptus." *Materials Today: Proceedings* 2 (1): 134–40. https://doi.org/10.1016/j.matpr.2015.04.038.

Nelson, Kim, and Theodora Retsina. 2014. "W w w. Ta p p i . o r G" 13 (5). Scientific Journal Edition. "Nanocellulose Markets"

Parit, Mahesh, Partha Saha, Virginia A. Davis, and Zhihua Jiang. 2018. "Transparent and Homogenous Cellulose Nanocrystal/Lignin UV-Protection Films." *ACS Omega* 3 (9): 10679–91. https://doi.org/10.1021/acsomega.8b01345.

Peretz, Roi, Elizaveta Sterenzon, Yoram Gerchman, Vinod Kumar Vadivel, Thomas Luxbacher, and Hadas Mamane. 2019. "Nanocellulose Production from Recycled Paper Mill Sludge

Using Ozonation Pretreatment Followed by Recyclable Maleic Acid Hydrolysis." *Carbohydrate Polymers* 216: 343–51. https://doi.org/10.1016/j.carbpol.2019.04.003.

Pflieger, Maryline, and Ana Kroflič. 2017. "Acute Toxicity of Emerging Atmospheric Pollutants from Wood Lignin Due to Biomass Burning." *Journal of Hazardous Materials* 338: 132–39. https://doi.org/10.1016/j.jhazmat.2017.05.023.

Ponnusamy, Vinoth Kumar, Dinh Duc Nguyen, Soon Woong Chang, Jeyaprakash Dharmaraja, Sutha Shobana, J Rajesh Banu, Rijuta Ganesh Saratale, and Gopalakrishnan Kumar. 2019. "A Review on Lignin Structure, Pretreatments, Fermentation Reactions and Biorefinery Potential." *Bioresource Technology* 271: 462–72. https://doi.org/10.1016/j.biortech.2018.09.070.

Qian, Yong, Yonghong Deng, Conghua Yi, Haifeng Yu, and Xueqing Qiu. 2011. "Solution Behaviors and Adsorption Characteristics of Sodium Lignosulfonate under Different PH Conditions." *BioResources* 6 (4): 4686–95.

Rafeek, Reisha, Christine V.F. Carrington, Andres Gomez, Derek Harkins, Manolito Torralba, Claire Kuelbs, Jonas Addae, Ahmed Moustafa, and Karen E. Nelson. 2019. "Xylitol and Sorbitol Effects on the Microbiome of Saliva and Plaque." *Journal of Oral Microbiology* 11 (1). https://doi.org/10.1080/20002297.2018.1536181.

Sadeghifar, Hasan, Richard Venditti, Jesse Jur, Russell E. Gorga, and Joel J. Pawlak. 2017. "Cellulose-Lignin Biodegradable and Flexible UV Protection Film." *ACS Sustainable Chemistry and Engineering* 5 (1): 625–31. https://doi.org/10.1021/acssuschemeng.6b02003.

Shimizu, Michiko, Tsuguyuki Saito, Hayaka Fukuzumi, and Akira Isogai. 2014. "Hydrophobic, Ductile, and Transparent Nanocellulose Films with Quaternary Alkylammonium Carboxylates on Nanofibril Surfaces." *Biomacromolecules* 15 (11): 4320–25. https://doi.org/10.1021/bm501329v.

Silva, Pablo A, Daniel Flórez-orrego, Silvio De Oliveira, François Maréchal, and Rubens Maciel. 2019. "Exergetic , Environmental and Economic Assessment of Sugarcane First – Generation Biorefineries" 99 (2): 67–81.

Trache, Djalal, M. Hazwan Hussin, M. K.Mohamad Haafiz, and Vijay Kumar Thakur. 2017. "Recent Progress in Cellulose Nanocrystals: Sources and Production." *Nanoscale* 9 (5): 1763–86. https://doi.org/10.1039/c6nr09494e.

Tyagi, Preeti, Lucian A Lucia, Martin A Hubbe, and Lokendra Pal. 2019. "Nanocellulose-Based Multilayer Barrier Coatings for Gas, Oil, and Grease Resistance." *Carbohydrate Polymers* 206: 281–88. https://doi.org/10.1016/j.carbpol.2018.10.114.

Zavarin, Eugene, Chieu Nguyen, John R Worster, and Edwin Romero. 1982. "Preparation Of Enzymatically Liberated Lignin From Naturally Brown-Rotted Wood." *Journal of Wood Chemistry and Technology* 2 (4): 343–70. https://doi.org/10.1080/02773818208085140.

Zhao, Yadong, Ayumu Tagami, Galina Dobele, Mikael E Lindström, and Olena Sevastyanova. 2019. "The Impact of Lignin Structural Diversity on Performance of Cellulose Nanofiber (CNF)-Starch Composite Films." *Polymers (Basel)* 11 (3). https://doi.org/10.3390/polym11030538.